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Is oyster broodstock feeding always necessary? A study using oocyte quality predictors and validators in *Crassostrea gigas*

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Abstract – The effect of providing algal food to female *Crassostrea gigas* broodstock sampled at a favourable time of year was investigated using pre-defined indices of oocyte quality and subsequent larval and post-larval performances. Broodstock were collected in the Aber Benoît estuary (Brittany, France) at the end of April 2002 and were divided into two groups, a starved control (S, 1 μm – filtered seawater) and a fed group (F, *ad libidum* algal mixture of *Isochrysis galbana* clone T-Iso and *Chaetoceros calcitrans*), maintained under these conditions for six weeks prior to fertilizations. For each conditioning type, two batches of larvae and post-larvae were reared from two identified females. Quality criteria were defined in terms of predictors (oocyte lipid contents, mature oocyte diameters, ovarian maturity and presence/absence of atresia) and validators (larval growth, algal consumption and time to settlement, and post-larval growth). Each female was considered as a treatment, and parametric and non-parametric ANOVAs were performed, where appropriate, on predictors and validators between each treatment. In both conditions, a surplus of oocytes was produced, in relation to expected market conditions. No effect of the conditioning treatment was detected either on oocyte quality predictors or on subsequent validators. It is likely that winter reserve accumulation is more important than trophic conditioning for successful gametogenesis in the productive Aber Benoît environment. Given the considerable cost of abundant feeding during the conditioning of broodstock collected at the end of winter and destined for gamete stripping (the prevalent practice in France), we suggest that feeding be minimal or suppressed altogether when the condition index is favourable.

Key words: *Crassostrea gigas* / Conditioning / Oocyte quality / Larvae / Post-larvae

Résumé – L'alimentation des huîtres génitrices est-elle toujours nécessaire ? Une étude utilisant des critères de prévision et de validation de la qualité des ovocytes chez l'huître *Crassostrea gigas*. L'effet de l'apport de nourriture algale à des géniteurs femelles de l'huître *Crassostrea gigas* échantillonnés à une période favorable de l'année a été étudié, utilisant des indices prédéfinis de qualité ovocytaire et de performances larvaires et post-larvaires subséquentes. Les géniteurs ont été collectés dans l'Aber Benoît (Bretagne, France) à la fin avril 2002 et ont été divisés en deux groupes, un groupe témoin non nourri (S, eau de mer filtrée à 1 μm) et un groupe nourri (F, mélange algal *ad libidum* de *Isochrysis galbana* clone T-Iso et *Chaetoceros calcitrans*), maintenus dans ces conditions durant six semaines avant les fécondations. Pour chaque type de conditionnement, deux lots de larves et de post-larves issus de deux femelles distinctes ont été élevés. Les critères de qualité ont été définis en termes de prédicteurs (teneurs en lipides des ovocytes, diamètres des ovocytes mûrs, maturité ovarienne et présence/absence d'atrésie) et de validateurs (croissance larvaire, consommation algale et temps à la métamorphose, puis croissance post-larvaire). Chaque femelle a été considérée comme un traitement, et des analyses de variance, ANOVA, paramétriques et non-paramétriques ont été effectuées, selon les cas, sur les prédicteurs et les validateurs entre chaque traitement. Pour les deux types de conditionnement, un surplus d'ovocytes a été produit, par rapport à la demande potentielle du marché. Aucun effet du régime de conditionnement n'a été détecté, ni sur les prédicteurs de qualité ovocytaire, ni sur les validateurs subséquents. Il semblerait que pour une gamétogenèse réussie, l'accumulation de réserves pendant l'hiver soit plus importante que le conditionnement trophique, dans l'environnement productif de l'Aber Benoît. Etant donné le coût considérable d'une alimentation abondante pendant le conditionnement de géniteurs prélevés à la fin de l'hiver et destinés au « stripping » (la pratique courante en France), nous proposons de réduire, voire même de supprimer l'alimentation pendant le conditionnement lorsque l'indice de condition est favorable.

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1 Introduction

The Pacific oyster, *Crassostrea gigas* (Thunberg 1793), is one of the most important cultured species, with a high value and strong market demand (Castagna et al. 1996), especially in France, where approximately 150 000 tons are produced every year since 1990, with significant export (Dauvin 1997). High reliance on wild spat collection in France is a potential weak point in oyster production. The role of hatcheries is thus increasing, with emphasis on the optimization of gamete quality (Massapina et al. 1999; Valdez-Ramirez 1999).

Oocyte quality is an important factor influencing subsequent larval survival and development (see Mann 1988 for bivalves in general; Gallager and Mann 1986 for *Crassostrea virginica* and *Mercenaria mercenaria*; Dorange et al. 1989; Le Pennec et al. 1990; Devauchelle and Mingant 1991 for *Pecten maximus*), and developing tools to evaluate oocyte quality is necessary. "Quality", however, is not a well-defined concept, variously characterized by oocyte cytological and ultrastructural aspects (Daniels et al. 1973; Valdez-Ramirez et al. 1999; Valdez-Ramirez et al. 2002), morphological features (Valdez-Ramirez 1999; Valdez-Ramirez et al. 1999) as well as physical (Gallager and Mann 1986), physiological (Valdez-Ramirez et al. 1999) and biochemical aspects (Whyte et al. 1987, 1990; Robinson 1992a; Ruiz et al. 1992; Valdez-Ramirez 1999; Valdez-Ramirez et al. 1999; Li et al. 2000; Caers et al. 2002; Hendriks et al. 2003), notably the lipid content and composition.

Although often employed in aquaculture studies, oocyte quality remains to be effectively and clearly defined. Obviously, those oocyte characteristics which are related to high fertilization success and good larval performance may be used as quality indicators. This functional definition of quality requires the linking of specific oocyte characters to ultimate culture performance indicators. Two different sets of characteristics may thus be proposed to define and quantitatively measure oocyte quality: (1) predictive criteria (predictors), which are oocyte characteristics associated with quality, and (2) validating criteria (validators), which are indices of larval and post-larval performances.

Potential candidates for oocyte predictor status are oocyte diameter (oocytes which remain small do not mature), structural integrity (i.e. no symptoms of atresia – Pipe 1987; Dorange and Le Pennec 1989; Le Pennec et al. 1991a), maturity status (pre-vitellogenic, vitellogenic, mature), and biochemical (especially lipid) content and composition. Extensive research has shown larval catabolism of pre-existing oocyte lipid prior to complete exotrophy (Barlett 1979; Lucas et al. 1986; Whyte et al. 1987, 1990, 1992; Lee and Heffernan 1991; Delaunay et al. 1992; Videla et al. 1998; Labarta et al. 1999; Lu et al. 1999; García-Esquivel et al. 2001).

Oocyte quality validators obviously include (1) time to metamorphosis and (2) larval growth, both of which have been shown to significantly affect spat growth (Collet et al. 1999). As (3) post-larval growth rate is strongly correlated with larval growth rate (Newkirk et al. 1977), it is also of interest as an oocyte quality validator, all the more so because it is a "long-range" validator, of particular interest to shellfish farmers. Algal consumption during larval rearing (4) may reasonably be considered to be a quality validator, under the assumption that larvae which feed well are likely to be in good condition.

Oocyte quality predictors and their subsequent validators may be expected to be influenced by broodstock diet, or more generally, by broodstock energetics. Thus some studies show that broodstock diet influences larval growth in *Ostrea edulis* (Berntsson et al. 1997) and in *Ostrea chilensis* (Wilson et al. 1996), and the conventional wisdom is that *C. gigas* broodstock require large amounts of food during hatchery conditioning, and that certain nutritional qualities are required for complete gametogenesis (Robert and Gérard 1999).

Recently, Caers et al. (2002), showed that despite having an influence on oocyte and larval fatty acid compositions (as had been previously reported – Robinson 1992a; Soudant et al. 1996a,b), there was no impact of *C. gigas* broodstock diet type on either egg size and lipid content, or on larval sizes and performances. Recent studies on several bivalve species, including *C. gigas,* did not detect any effect of lipid-enriched diets on oocyte sizes and lipid contents, but rather showed increased oocyte numbers (Hendriks et al. 2003; Nevejan et al. 2003). Chávez-Villalba et al. (2003a,b) went beyond diet type to include a non-fed condition during their *C. gigas* broodstock conditioning, and no effect of female broodstock starvation was observed in some indicators of larval performance (growth, D larval yield). These results seem to contradict a considerable body of earlier work. Wilson et al. (1996) observed a significant relation between broodstock starvation and lower growth rates (longer incubation times) and survival, and longer time to metamorphosis and fixation in *Ostrea chilensis*. In addition, Robinson (1992b) detected differences in fecundity and in larval and post-larval performances between oysters subjected to different conditioning diets, including starvation. More recently, Uriarte et al. (2004) showed that protein and lipid enrichments in conditioning diets improved larval performances in *C. gigas*. The surprising results of Chávez-Villalba et al. (2003a,b) thus require confirmation, not only because they are counter-intuitive, but also because of the considerable resources spent in hatcheries on algal production for conditioning. In France, as elsewhere, conditioning of *C. gigas* is performed on adults collected from the wild and gametes are stripped (Castagna et al. 1996); hence broodstock cannot be "recycled" for subsequent conditionings and fertilizations. Furthermore, the results of Caers et al. (2002) and Chávez-Villalba et al. (2003a,b) require longer-range performance confirmation, since their cultures did not go beyond 7 and 16 days, respectively.

With a view to resolving whether abundant feeding (current practice) or no feeding during conditioning may influence oocyte quality, we therefore tested the effects of two conditioning treatments, abundant feeding and starvation, on a set of clearly-identified *C. gigas* oocyte quality predictors (lipid content, mature oocyte diameters, oocyte maturity, and presence/absence of atresia) and validators (larval growth, algal consumption and time to metamorphosis, and post-larval growth).

2 Materials and methods

2.1 Oyster conditioning

Sixty adult oysters were collected from the Aber Benoît estuary (48°35 N; 4°37 W, Brittany, France) at the end of April 2002, and transferred to the nearby Argenton experimental hatchery (IFREMER). Oysters were divided into two conditioning groups of 30 individuals each: (1) a conditioning labelled "Fed" (F), in which oysters were abundantly fed with a commonly-used mixture of two micro-algae species, *Isochrysis galbana* (clone T-Iso) and *Chaetoceros calcitrans,* using the protocol of Chávez-Villalba et al. (2002), and (2) a conditioning labelled "Starved" (S), in which oysters were maintained without particulate food in running, 1 μm – filtered sea water (FSW), for a period of six weeks prior to gamete stripping. Female oysters were assumed to be at the beginning of their gametogenesis when conditioning began at the end of April (Chávez-Villalba et al. 2001). In order to ascertain that oocyte quality predictors and validators pertained to the same females, two females from each treatment were used for fertilizations, and the resulting four larval cultures were carried out and monitored as detailed below.

2.2 Predictors: Oocyte characteristics

Stereology

Stereology was used to quantify the proportions of each female gamete stage immediately prior to gamete stripping in the two conditions (Weibel et al. 1966; Freere 1967; Briarty 1975; Beninger 1987; Morvan and Ansell 1988; Mayhew 2000; Gosling 2003). In addition to their role as a gamete quality predictor, the stereological counts also furnished a quantitative measurement of the overall state of the gonad in each of the females used for fertilizations, so that it was possible to assess whether or not all females used for gamete stripping were in the same state of readiness. A standard biopsy (approx. 5 mm^3) of the gonad was performed for each female at the junction of the gills and the labial palps (Morales-Alamo and Mann 1989) and fixed in aqueous Bouin's solution. After rinsing for a minimum of 10 h and dehydration in an ascending ethanol – Histo-Clear® series, the biopsies were embedded in paraffin and sectioned at 7 μm. Sections were stained using a modified Masson's trichrome protocol, with trioxyhematein, acid fuschin and fast green (Martoja and Martoja-Pierson 1967; Gabe 1968) for 1 min 30 s, 5 s, and 1 min respectively.

Three female gonad cell categories were identified and counted using a microscope projector and the subsequent volume fractions were calculated: mature oocytes, immature (pedunculate) oocytes and connective tissue. Nine counts were performed for each female, on surfaces of known area, using a 9×9 point matrix.

Oocyte diameters

The diameters of thirty mature oocytes from each female were measured from whole stripped gametes (see below). The free oocyte sizes supplied by this procedure (as opposed to diameters obtained from histological sections, which present an unknown amount of bias due to shrinkage), are thus related to cell volume ($V = 4/3\pi r^3$), and hence to amount of cell content. Due to their irregular shape, pedunculate oocytes were not used for these measurements.

Atresia

Characteristic signs of oocyte atresia (thickened and/or clear cell envelope, irregular shape) were monitored in the histological sections, while performing stereological counts.

Lipid content

To estimate lipid quantities in oocytes for each female, samples of oocytes were collected on 20 μm mesh in FSW after gonad stripping (see below). Oocyte concentrations were estimated using a particle counter (Coulter Multisizer II®). The oocytes were then rinsed, frozen and stored at −80 °C prior to analysis. Oocyte total lipid contents were subsequently quantified using the micro-analytical method of Marsh and Weinstein (1966), with triolein as a standard (rather than tripalmitin, given the preponderance of unsaturated fatty acids in bivalve oocytes – Trider and Castell 1980; Whyte et al. 1990; Marty et al. 1992; Napolitano et al. 1992; Berntsson et al. 1997; Utting and Millican 1997). In preliminary trials, there was a very close relationship between absorbance at 375 nm and triolein weight ($r^2 = 0.992$).

2.3 Fertilizations, larval and post-larval cultures

Fertilizations, larval and post-larval cultures were conducted at the Argenton experimental hatchery. Two females of similar size per conditioning type were selected (size range 95−105 mm, long axis). Oocytes were collected by stripping in FSW (Allen and Bushek 1992; Castagna et al. 1996). Each oocyte pool was divided into two aliquots: a small portion was fixed in 10% buffered formalin, pH 7.2 in order to measure oocyte diameters, and the rest was used for fertilization.

Pooled sperm in FSW originating from two males from the F conditioning was used for fertilizing batches of oocytes, to ensure that male broodstock nutritional state did not interfere with fertilization success. Spermatozoa were collected by stripping the male gonad and diluted in 1 L of FSW. The two males were chosen on the basis of spermatozoon mobility under light microscopy.

Oocytes were counted using a profile projector and approximately 5×10^6 oocytes for each female were distributed in four fertilization beakers at 21 °C. Oocytes were then fertilized by addition of 50 ml of the spermatozoa pool. Samples were removed and observed during fertilization, to verify the presence of 8 to 10 spermatozoa around each oocyte (Valdez-Ramirez 1999) and to monitor fertilization (Robert and Gérard 1999). As the gonads of broodstock females were biopsied for lipid determinations and histological data, it was not possible to obtain total oocyte estimations.

After fertilization, larval cultures were maintained in 150 L tanks of aerated FSW. Larval concentrations were equalized to 300 000 larvae per tank (2 larvae ml^{-1}) at day 2. All larvae were fed a 1:1:1 mixture of *Pavlova lutheri*, *Isochrysis galbana*

(clone T-Iso) and *Chaetoceros calcitrans* (PTC diet – Robert and Gérard 1999; Robert et al. 2001). The rearing tanks were emptied, washed and refilled every 2–3 days. Water temperature was maintained at 22 °C at the beginning of larval culture, gradually raised to 24–25 °C on day 6, and maintained at this temperature until settlement (Robert and Gérard 1999).

At day 20, eyed larvae were left to metamorphose and fix onto finely-broken oyster shells in rectangular PVC sifters of 140 μm diagonal mesh placed in raceways (density approx. 60 000 larvae per sifter). FSW (5 μm) was distributed continuously in the raceways with PTC diet. At day 34, fixed post-larvae were isolated from free broken oyster shells by sifting (350 μm diagonal mesh) and replaced in 150 μm sifters. Post-larval cultures were stopped at day 38.

2.4 Validators: Larval and post-larval performances

Algal consumption

Algal consumption per larva and per batch was monitored in order to verify condition and feeding aptitude of larvae and also (together with growth data) as a relative index of mortality in the two conditioning treatments, by counting algal concentrations in the rearing tanks after feeding larvae and just before water changes, using the particle counter. In essence, batches with closely similar algal consumptions and larval growths would have similar mortalities. Two counts of 50 μl samples were performed for each batch, summed and expressed as concentration per ml.

Growth

During larval and post-larval cultures, samplings were performed to estimate larval and post-larval growth in the four batches. Larvae and post-larvae were regularly sampled and fixed in 10% buffered formalin, pH 7.2. Thirty individual measurements of the longest shell axis were carried out for each larval or post-larval sample, using a compound microscope fitted with an ocular micrometer.

The sampling schedule was the following: days 3, 6, 8, 10, 13, 15, 17 and 20 for larval cultures and days 27, 34 and 38 for post-larval cultures.

Time to metamorphosis

From day 17, particular attention was paid to the appearance of the larval eyespot, and to larval behaviour and position in the culture containers. At day 20, the majority of the larvae of all four batches were eyed and showed substrate search ("swimming-crawling") behaviour. Transfer to new culture containers was effected as described above, and as nearly all larvae had disappeared from the water column on day 21, the time to metamorphosis was determined as day 20.

2.5 Statistics

Since a single female generates considerable oocyte quality data (e.g. mean oocyte diameters, stereological data), as well as an entire larval rearing batch, for statistical purposes each female was considered as a treatment, and one-way ANOVAs were performed on oocyte diameters, stereological counts and shell sizes. Hence, lack of significant difference between females of the same conditioning treatment is to be expected, whereas a significant difference between females of the F and the S condition would denote a difference related to the condition. Normality and heteroscedasticity were verified for each statistical test, and percentage data were arcsine transformed to eliminate bias from variance linkage to the mean. In data sets where non-normality persisted, a non-parametric one-way ANOVA was performed (Sokal and Rohlf 1995). All statistical treatments were carried out at a significance level of $\alpha = 0.05$.

As our experimental design required quality predictors and validators to be measured on the products of the same individual females, and it was not possible to anticipate subsequent larval mortality, the amount of oocytes available for each predictor was voluntarily limited. In order to maximize fertilizations for larval cultures, oocyte lipid content was therefore determined only once for each female, hence obviating statistical comparisons for this characteristic.

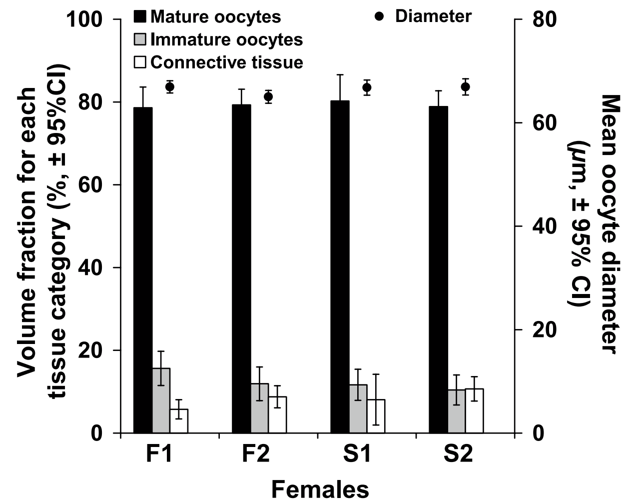

Fig. 1. Tissue volume fractions of the gonad in the 4 females: Fed F1, F2 and Starved S1, S2; vertical bars are 95% confidence intervals ($n = 9$). Filled circles: Mean oocyte diameters for the 4 females: F1, F2, S1 and S2. Vertical bars are 95% confidence intervals ($n = 30$).

3 Results

3.1 Predictors: Oocyte characteristics

Stereology and oocyte diameters

Gonad cell volume fractions of all four females appeared to be homogeneous, as shown in Figure 1. There were no significant differences between females for mature oocytes ($p = 0.948$), pedunculate oocytes ($p = 0.278$) and connective tissue ($p = 0.119$). The four females showed identical maturation states: mature oocytes represented the great majority of the tissue volume percentages (means and range of 95% confidence intervals CI) 79 to 80% ± 4 to 6%; pedunculate oocytes represented a smaller tissue fraction (10 to 16% ± 4%

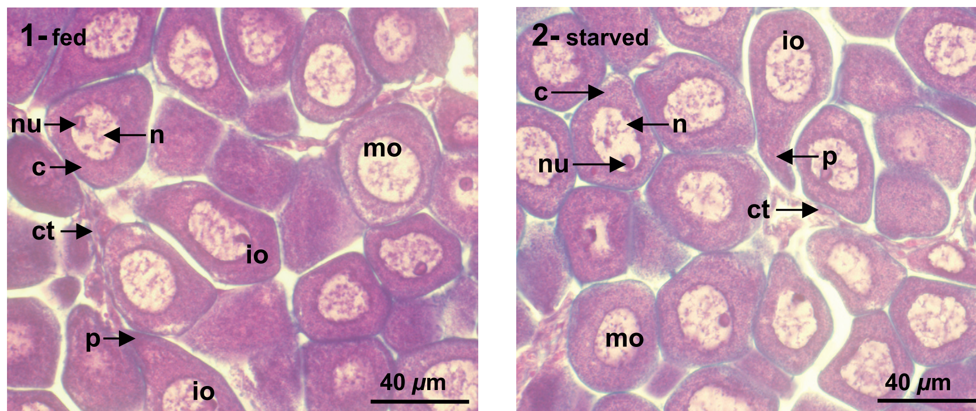

Fig. 2. Photomicrographs of histological sections of female gonads of both the "F-Fed" and "S-Starved" conditioning treatments: illustration of maturation state and absence of atresia. Modified Masson's trichrome protocol. **1** – Female F2. **2** – Female S2. **c**: cytoplasm, **ct**: connective tissue, **io**: immature (pedunculate) oocytes, **mo**: mature oocytes, **n**: nucleus, **nu**: nucleolus, **p**: peduncule.

Table 1. Oocyte lipid contents for the two conditioning treatments: Fed (F) and Starved (S) (ng equivalent triolein oocyte^{-1}). Values correspond to means of three spectrophotometric readings, dispersion nil.

Treatment	Fed		Starved	
Females	F1	F2	S1	S2
Oocyte lipid content	4.7	9.0	22.6	3.9

and connective tissue was also less present (6 to 11% ± 2 to 6% – see Fig. 2). All females therefore presented oocyte populations with similar maturity profiles, regardless of conditioning treatment, and were thus in the same maturation state when fertilization was carried out (Figs. 1 and 2).

Mature oocyte mean diameters are presented for each female (Fig. 1). Values were not significantly different (ANOVA, $p = 0.152$), either between conditioning types or between individuals, and varied only slightly around a mean of 65.0 to 66.9 μm ± 1.2 to 1.6 μm (means and range of confidence intervals CI).

Atresia

No evidence of oocyte atresia was detected in any of the histological sections examined (Fig. 1), and females of both the F and S conditioning treatment showed the same gonad profile: mature oocytes occupying the majority of the sectioned gonad, no atresia observed (Fig. 2).

Oocyte lipid contents

Oocyte lipid contents are presented for the four females (Table 1). The range of values for all four females overlap completely, and the difference between the two values of the F condition is much smaller than the corresponding difference in the S condition. It should be noted that the highest value (22.6 ng oocyte^{-1}) was obtained for female S1; conversely, the lowest value was obtained for female S2 (3.9 ng oocyte^{-1}) (Table 1).

3.2 Validators: Larval and post-larval performances

Algal consumption and mortality

The daily algal consumptions per larva and per batch throughout the larval phase from day 6 to day 20 are shown (Fig. 3). Algal consumption increased fivefold from day 6 to day 16 (2.33×10^9 cells day^{-1} to 12.35×10^9 cells day^{-1} per batch). From day 16 to day 20 algal consumption decreased 2.5 fold (12.35×10^9 cells day^{-1} to 4.40×10^9 cells day^{-1} per batch), corresponding to the onset of "swimming-crawling" behaviour just prior to metamorphosis and settlement (Gerdes 1983) (Fig. 3). Total daily algal consumptions were quite similar in larvae from both conditioning treatments and all four batches throughout the larval phase. Since larval growths were so similar (see below), the close values of the per batch algal consumptions indicate a uniform mortality in all cultures.

Larval growth and time to metamorphosis

Mean larval shell sizes did not differ significantly between the two conditioning treatments or among batches ($p = 0.059$ at day 6; $p = 0.337$ at day 15; $p = 0.870$ at day 20). The growth curves of all four larval cultures were remarkably similar in both shape and absolute values from day 0 to day 20 (Fig. 4). Growth was not depressed in larvae produced by S females. For both broodstock conditioning types, growth was interrupted from day 17 to day 20.

Eyed larvae were allowed to fix and metamorphose at day 20 at an approximate size of 358 μm, and the mean larval growth rate from day 0 to day 20 was approx. 14.6 μm day^{-1} (Fig. 4).

In light of the decreased growth rates, total algal consumptions, "swimming-crawling" behaviour, and absence of swimming larvae after day 20, time to metamorphosis was therefore taken to be day 20 (Fig. 4).

Post-larval growth

As was observed for the larval cultures, post-larval growth curves of all batches were similar in form and values for the three sampling dates (27, 34 and 38 days after fertilization,

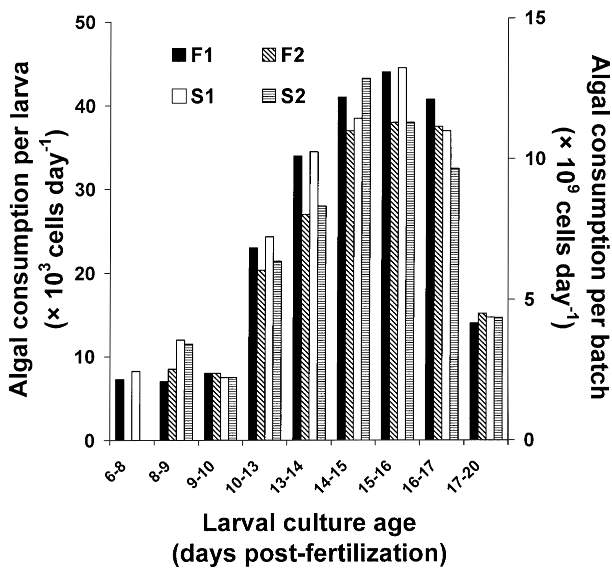

Fig. 3. Algal consumption per larva ($\times 10^9$ cells day^{-1}) and per batch ($\times 10^3$ cells day^{-1}) from day 6 to day 20 for the four larval batches: F1, F2, S1 and S2. No confidence intervals (n = 1).

Fig. 4). Mean shell sizes (± range of confidence intervals) were:

887 to 914 μm ± 29 to 44 μm at day 27

1479 to 1528 μm ± 63 to 76 μm at day 34 and

2131 to 2241 μm ± 123 to 159 μm at day 38.

There were no significant mean size differences between the cultures at any of the three dates ($p = 0.749$, $p = 0.768$ and $p = 0.689$ respectively).

4 Discussion

The results of the present study show that feeding during broodstock conditioning did not affect oocyte quality or subsequent larval and post-larval performances, based on the set of predictors and validators defined herein. The observations of Chávez-Villalba et al. (2003a,b) are thus confirmed and extended to spat cultures up to 38 days. The homogeneous quality of *Crassostrea gigas* oocytes in these two conditions suggests that oocyte quality is primarily dependant on the reserves accumulated by broodstock prior to conditioning.

Adult reserves for oogenesis are chiefly comprised of glycogen, transformed to lipid reserves within oocytes during vitellogenesis (Gabbott 1975; Bayne et al. 1982; Deslous-Paoli et al. 1982; Beninger and Lucas 1984; Lubet and Mann 1987; Ruiz et al. 1992; Pazos et al. 1996; Thompson et al. 1996; Berthelin et al. 2000; Heude Berthelin et al. 2003). The lipid content of the *C. gigas* oocytes, although somewhat variable among the individual broodstock females (4−22 ng oocyte^{-1}), includes the range of values previously reported (7−8 ng oocyte^{-1} – Caers et al. 2002), and seems to suffice for excellent larval and post-larval performance. Contrary to other bivalves, *C. gigas* oocyte quality and subsequent performance do not appear to be influenced by specific dietary

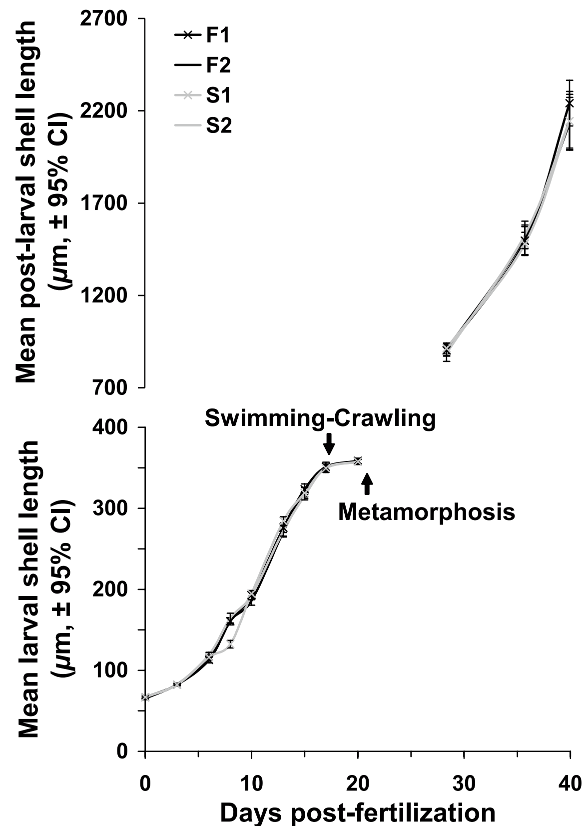

Fig. 4. Mean shell size evolution for larvae and post-larvae generated by the four broodstock females: Fed F1, F2, and Starved S1, S2 during the larval and post-larval cultures. The day 0 size corresponds to mean oocyte diameter. Vertical bars are 95% confidence intervals (n = 30).

lipid fatty acid supplementation, so this need not be a concern for hatchery operators (Caers et al. 2002; Hendriks et al. 2003; Nevejan et al. 2003).

To what point beyond fertilization oocyte quality affects performance is not certain. The progression from endotrophy to mixotrophy and finally exotrophy has been studied in *Mytilus edulis* by Lucas et al. (1986); use of oocyte reserves continued up to day 8 after fertilization. The scant information available for *C. gigas* yields a range of ages for the onset of larval feeding: 24−30 h (Lucas and Rangel 1983), 24−48 h (Langdon and Newell 1996; Newell and Langdon 1996), 90 μm (approx. 4 days in this study) (Strathmann and Leise 1979). It is not known up to what age oocyte reserves contribute to larval nutrition, but Gallager et al. (1986) found vitellin lipid droplets in larvae of *C. gigas* up to 4 days after the initiation of feeding. In any event, oyster growers are likely to prefer long-range validation of oocyte quality.

It should be noted that the very good larval and post-larval performance of the four broodstock females was obtained after sampling from the Aber Benoît estuary at the beginning of spring, followed by conditioning in the hatchery. Previous studies have shown that for *C. gigas* reared in France, sampling and conditioning at other seasons results in reduced fecundity, especially in winter, where fecundity is reduced to 1/10−1/5 that of spring conditioning (Chávez-Villalba et al. 2002, 2003a,b). Spring conditioning thus appears to be the

optimal configuration for *C. gigas*, and this is supported by the observation that broodstock diet type does not influence oocyte diameter, number, or lipid content, larval or post-larval growth when conditioning takes place in spring (Robinson 1992b; Caers et al. 2002). The Aber Benoît estuary, from which the broodstock of the present study were obtained, is known to be trophically rich for oyster growing (Fleury et al. 2001), allowing accumulation of reserves prior to oocyte vitellogenesis.

The optimal result obtained with spring conditioning is a notable constraint to one of the most important objectives of hatchery production: a regular supply of spat throughout the year. To maintain production levels, it is thus necessary to condition more females for fertilization outside the spring period; this will, of course, entail increased costs.

The results of the present study suggest that previously-stored reserves are used for vitellogenesis in *C. gigas*, as is supposed for bivalves generally (Gabbott 1975; Bayne 1976). It is interesting to note, however, that *Loripes lucinalis*, which harbours chemoautotrophic endosymbionts, appears to require near-simultaneous particulate food input during oogenesis (Le Pennec et al. 1995) and that *Pecten maximus* seems to be able to directly transfer metabolites from the digestive system to gametes (Le Pennec et al. 1991a,b; Beninger et al. 2003).

Although the present study confirms the importance of reserves accumulated prior to conditioning, for oocyte quality, there are indications that under conditions of food limitation, female oyster broodstock maintain a constant oocyte quality but reduce the number of oocytes produced (Bayne 1976; Chávez-Villalba et al. 2003a). As all four females of the present study produced sufficient oocytes for market conditions, this was not a limiting factor.

It is tempting to conclude that broodstock obtained in spring need not be fed during conditioning, if gametes are to be obtained by stripping, since this will result in a substantial economic saving for the hatchery. This is of course possible if the winter habitat provides enough food for the constitution of abundant reserves, as reflected in the condition index of oysters from the Aber Benoît estuary (Fleury et al. 2001). Condition index (Lucas and Beninger 1985) monitoring would be a particularly appropriate means of establishing whether or not broodstock are to be fed during conditioning. Furthermore, in the special case of male tetraploid oysters, which are quite fragile (Longwell and Stiles 1996; Pouvreau, personal communication), used in the increasingly popular production of triploids, it may not be feasible or prudent to monitor condition indices or to suppress feeding during conditioning.

The second major conclusion of the present study is that the selected set of oocyte quality predictors is valid, i.e. confirmed by both short (larval growth and algal consumption) and long-range validators (time to metamorphosis, post-larval growth). In the present study, the excellent performance of larvae and post-larvae indicates the good levels of the quality predictors: the level of algal consumption is equal to or higher than that previously reported to be required for healthy cultures (Robert and Gérard 1999), growth and time to metamorphosis are consistent with good performance in previous studies (Gerdes 1983; Robert and Gérard 1999), and the indirect measure of larval mortality is virtually nil. The usefulness of these predictors will depend on the hatchery context. If broodstock are stripped for gametes (as is current in France and widespread practice for *C. gigas* – Castagna et al. 1996), all of the predictors may be used (although information from histology will only be available within 2−3 days). If broodstock are induced to spawn and saved for future use or if a broodstock selection program is in effect, only oocyte diameter can be used.

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