

Acquisition of particle processing capability in the oyster *Crassostrea gigas*: ontogeny of the mantle pseudofeces rejection tracts

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ABSTRACT: Effective particle processing by oysters, in their typically turbid habitats, requires an efficient mechanism of pseudofeces rejection, accomplished by the characteristic oyster mantle rejection tracts. The ontogenetic development of these tracts was studied by means of scanning electron microscopy (SEM) for late larval, postlarval, and juvenile *Crassostrea gigas* (Thunberg 1793), using hatchery-reared specimens. Pediveliger larvae had a marginal mantle rejection tract, which becomes the inner fold of the 3 mantle folds in postlarval and adult oysters. An ephemeral, secondary marginal ciliary tract was observed in 1.30 mm early postlarvae; this was absent in later postlarval stages. Specimens 2.70 mm in size had a 'tractless' mantle surface, constituting a potentially critical stage with respect to pseudofeces rejection and hence particle processing. The organization of the much more complex adult system, consisting of multiple radial tracts and a marginal collecting tract comprised of 2 contiguous sub-tracts, was not complete until specimens were 10 to 24 mm. The complete re-organization of the mantle rejection system suggests that this is a phylogenetically new character in the Ostreidae. Furthermore, it points to an additional potentially critical event in the acquisition of particle processing capability, especially when considered in the light of previous data on gill development in this species, notably for individuals recruiting in natural habitats.

KEY WORDS: *Crassostrea gigas* · Larvae · Postlarvae · Juveniles · Mantle · Rejection · Pseudofeces

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INTRODUCTION

Suspension-feeding bivalves, and especially the most conspicuous species such as oysters, play important roles in seston dynamics (Cloern 1982, Dame 1993, Gili & Coma 1998, Newell 2004) and more generally as physical and functional ecosystem engineers (see review by Ruesink et al. 2005). The anthropogenically induced decline in natural oyster populations (Ruesink et al. 2005) is contemporaneous with an opposite trend in increasing culture production. Oysters are the leading bivalve aquaculture species worldwide, and 95% of the landings are from culture operations; approximately one-third of this is due to the increasingly ubiquitous species *Crassostrea gigas* (Gosling 2003).

The mechanisms involved in oyster suspension-feeding have been investigated using a variety of modern

approaches, chiefly centred upon the roles of the gills and labial palps (Newell & Jordan 1983, Ward et al. 1994, 1998, Beninger & Dufour 1996, Cognie et al. 2003, Beninger et al. 2005). Ingestion volume control and qualitative selection both produce pseudofeces, and the transport and expulsion of pseudofeces is therefore essential to prevent fouling and unnecessary re-treatment in the suspension-feeding system. The role of the mantle rejection tracts in this regard was underscored in adult oysters (Beninger & Veniot 1999), but it is not known whether the vulnerable postlarval and juvenile stages are equipped to evacuate pseudofeces in this way. While recent advances have been made in the understanding of gill development and its potential impact on particle processing in early life stages (Chaparro et al. 2001, Cannuel & Beninger 2006), we have no knowledge concerning the development of the equally

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important pseudofeces evacuation mechanisms which allow oysters to exploit their typically turbid habitats, both in natural populations and as seed oysters in culture operations. Indeed, the bivalve mantle in general has until recently been poorly studied with respect to particle processing mechanisms (Beninger & Veniot 1999, Beninger et al. 1999). In oysters, descriptions of mantle development are typically either absent (Cole 1938, Galtsoff 1964, Hickman & Gruffydd 1971, Moor 1983) or quite brief, being limited to summary descriptions of cell types and mantle folds (Elston 1980, 1996). Indeed, in his reference monograph, Galtsoff (1964, p. 368) states that: 'Many phases of larval-metamorphosis, especially of the *Crassostrea* group of oysters, are inadequately known and need to be more critically studied. With advances in the technique of artificial rearing of oyster larvae, this gap in the knowledge of oyster biology may soon be filled'. More than 40 years later, with the recent notable exceptions of Chaparro et al. (2001) for *Ostrea chilensis* and Cannuel & Beninger (2006) for *Crassostrea gigas* concerning gill development, his prediction is still largely unfulfilled.

In the present study, we document the development of the mantle rejection tracts in late larval, postlarval and juvenile *Crassostrea gigas*, and relate this to the previously described concomitant development of the gills (Cannuel & Beninger 2006), and to the previously documented adult condition (Beninger & Veniot 1999).

MATERIALS AND METHODS

Developmental studies of post-metamorphic bivalves are hampered by a lack of precise terminology with respect to early benthic life stages. The term 'postlarva' could refer to any post-metamorphic individual (no exact upper size or age limit), whereas the term 'juvenile' is restricted to sexually immature individuals. Size or age at sexual maturity are variable, and at any rate impossible to ascertain in specimens which must be anatomically intact for developmental studies. 'Spat' is a common term which is as imprecise as 'postlarva' and conveys no biological information. The results of Cannuel & Beninger (2006) and the present work lead us to adopt the following convention: 'postlarvae' are individuals from settlement to 2.70 mm (inclusive), whereas 'juveniles' are individuals up to a size of 24 mm, irrespective of sexual maturity status.

Larval and postlarval cultures. Fertilizations, larval and postlarval cultures of *Crassostrea gigas* were obtained and reared as previously described (Cannuel & Beninger 2006). Briefly, oocytes and spermatozoa were obtained in April 2002 by gonad stripping of adult oysters previously conditioned for 6 wk. Larval cultures were started at an initial density of 30 to 35 larvae ml⁻¹,

in 150 l rearing tanks of aerated 1 µm filtered seawater (FSW). At Day 2, larval concentrations were reduced to 2 larvae ml⁻¹. Larvae were fed ad libitum with a 1:1:1 mixture of *Pavlova lutheri* (Droop) Green 1975, *Isochrysis aff. galbana* (Parke 1949, clone T-Iso Green) and *Chaetoceros calcitrans* (Paulsen) Takano 1968 PTC-diet (Robert & Gérard 1999). Initial culture temperature was 22°C, gradually raised to 24–25°C on Day 6, and maintained at this temperature to settlement (Robert & Gérard 1999).

At Day 20, the competent larvae were transferred to rectangular PVC sifters of 140 µm diagonal mesh placed in raceways and filled with finely crushed oyster shells, at a density of 60 000 larvae per sifter, for settlement, metamorphosis and postlarval rearing. Postlarvae were fed continuously with a PTC diet in 5 µm FSW. Raceways and sifters were washed first 1 wk after settlement and then every 2 to 3 d. Postlarval rearing was stopped at Day 36 (2.70 mm in shell size; Table 1).

Juvenile collection. *Crassostrea gigas* juveniles (>2.70 mm) were obtained from the Vendée Naissain company in September and December 2003, where they had been fed with *Skeletonema costatum* (Greville) Cleve 1873. Four stages were studied: 2.85, 7.50, 10.06 and 23.83 mm, corresponding to the mean shell lengths of 4 commercial sifter-sizes (Table 1). For convenience, the 2 larger shell sizes will be rounded off to 10 and 24 mm in the text.

Scanning electron microscopy (SEM) preparation. To ensure valve unlocking, tissue penetration and optimal fixation of relaxed structures, specimens were first narcotized in ascending concentrations of MgCl₂, up to 7.5% in narcotization beakers (Veniot et al. 2003, Cannuel & Beninger 2006). Specimens were then fixed in slightly hyperosmotic 2.5% glutaraldehyde in 0.2 M buffered sodium cacodylate for a minimum of 48 h and processed for drying with hexamethyldisilazane (HMDS; Nation 1983, Cannuel & Beninger 2006). The smaller specimens (larvae, postlarvae and juveniles up to 7.50 mm) were then mounted on SEM stubs using

Table 1. *Crassostrea gigas*. Age and mean shell length, standard deviation (SD), and sample (n) for each sampling

| Age | Mean shell length (mm) | SD | n |
|-------|------------------------|------|----|
| 15 d | 0.29 | 0.03 | 6 |
| 20 d | 0.33 | 0.02 | 7 |
| 22 d | 0.42 | 0.04 | 9 |
| 29 d | 1.30 | 0.13 | 5 |
| 36 d | 2.70 | 0.57 | 5 |
| 10 wk | 2.85 | 0.11 | 3 |
| 13 wk | 7.50 | 0.50 | 5 |
| 16 wk | 10.06 | 1.16 | 30 |
| 22 wk | 23.83 | 2.62 | 30 |

double-sided adhesive tape, and a fine tungsten needle was used to remove 1 valve, allowing visualization of internal organs. For larger specimens (7.50 to 24 mm) or detailed observation of organs, soft parts were removed from the shell before mounting on a stub, allowing optimal coating and subsequent visualization of the mantle surface. Samples were then sputter-coated with gold and observed with a scanning electron microscope (JEOL 6400).

RESULTS

Given the highly modified anatomy of oysters, compared to most other bivalves, it is important to establish unambiguous anatomical terms at the outset. Although the recommendations of Stasek (1963) regarding axis designation in relation to hinge location are quite satisfactory for the majority of bivalves, in species characterized by extensive rotation of organs accompanying the monomyarian condition, it is much easier to understand axis designations with respect to organ location. We therefore use the term 'anterior' to designate the extremity closest to the mouth, while the term 'ventral' is used to designate the extremity closest to the infra-branchial periphery. The terms 'posterior' and 'dorsal' refer to the opposites of these extremities.

In the present study, it is also necessary to distinguish the various pallial ciliary tracts and the developing mantle folds, which may also be ciliated, at the periphery of the mantle. The following terms will be used in the present study:

Marginal ciliary tract: a densely ciliated band, extending antero-posteriorly along the ventral mantle margin. It may be comprised of distinct anterior and posterior segments in the definitive condition.

Radial ciliary tracts: densely ciliated bands extending centrifugally on the mantle infrabranchial surface from the gill region to the mantle edge.

Mantle fold: any of the folds which appear at the ventral mantle edge. We use the term fold to avoid ambiguity with respect to the right and left pallial lobes. There are 3 such folds in the adult condition: inner, middle, and outer folds. The trough between the middle and outer folds is the periostracal groove.

Future inner fold: the larval marginal ciliary tract, which undergoes folding in the postlarvae to become the inner fold, which is retained in the adult condition.

Future middle fold: the outermost portion of the larval mantle inner fold, which becomes the middle fold after the folding of the larval future inner fold.

Future outer fold: the outermost larval mantle fold, which is retained in the adult condition.

As detailed below, illustrated in the micrographs (Figs. 1 to 3) and summarized in Fig. 4, 4 successive

stages of mantle ciliation were distinguished in the course of development of *Crassostrea gigas*: (1) Stage 1 presented a single, well-ciliated larval marginal rejection tract in 0.29 to 0.33 mm pediveliger larvae. This tract folds to become the inner mantle fold in postlarvae. (2) Stage 2 presented a new marginal ciliary tract in 1.30 mm postlarvae, situated further inward from the mantle edge in comparison to the larval marginal ciliary tract. (3) Stage 3 presented a transitional phase with randomly scattered sparse tufts of simple cilia over the entire mantle surface (2.70 mm postlarvae). (4) Stage 4 presented well-ciliated multiple radial rejection tracts and a new marginal collecting tract, consisting of anterior and posterior segments, in juveniles from 2.85 mm in shell size to the adult condition.

Tract dimensions and ciliary lengths reported in the present work were obtained directly from the micrographs; shrinkage due to specimen preparation was assumed to be uniform (Gusnard & Kirschner 1977), and estimated at 15 to 20% in bivalve ciliated epithelia (Beninger et al. 1999).

Reporting of bivalve developmental stages is usually done with respect to shell sizes (Beninger et al. 1994, Veniot et al. 2003), since there is a closer correspondence between size and development than between age and development (Veniot et al. 2003). In the present work, developmental stages are therefore given in terms of shell sizes; corresponding ages are presented in Table 1.

Stage 1. Pediveliger larvae: the marginal rejection tract

The mantle surface was unciliated in 0.29 mm larvae except on the future inner and middle folds. The cilia were especially well developed on the future inner fold, constituting the larval marginal ciliary tract, which extended posteriorly from the buccal region to mid-circumference (Fig. 1A). Near the mouth, this tract was ~4 to 6 μm in width, and composed of both simple (~2 to 5 μm in length) and apparently grouped (~5 to 8 μm in length) cilia (Fig. 1B). Most posteriorly, the ciliary tract was wider (~10 μm) and abundantly ciliated with grouped cilia (~7 to 12 μm) (Fig. 1C). Ventralward beating of these grouped cilia was inferred from cilia orientation on SEM micrographs.

The larval mantle marginal ciliary tract increased in size to ~15–18 μm in 0.33 mm pediveliger larvae, and consisted of long (~7.5 to 11 μm) grouped cilia, with short (~3 to 5 μm) cilia bordering the future middle fold (Figs. 1D–E & 4A). This tract persisted in 0.42 mm postlarvae, just after metamorphosis (Fig. 1F). Tufts of simple cilia (~10 μm), situated slightly dorsal and parallel to the persistent larval marginal

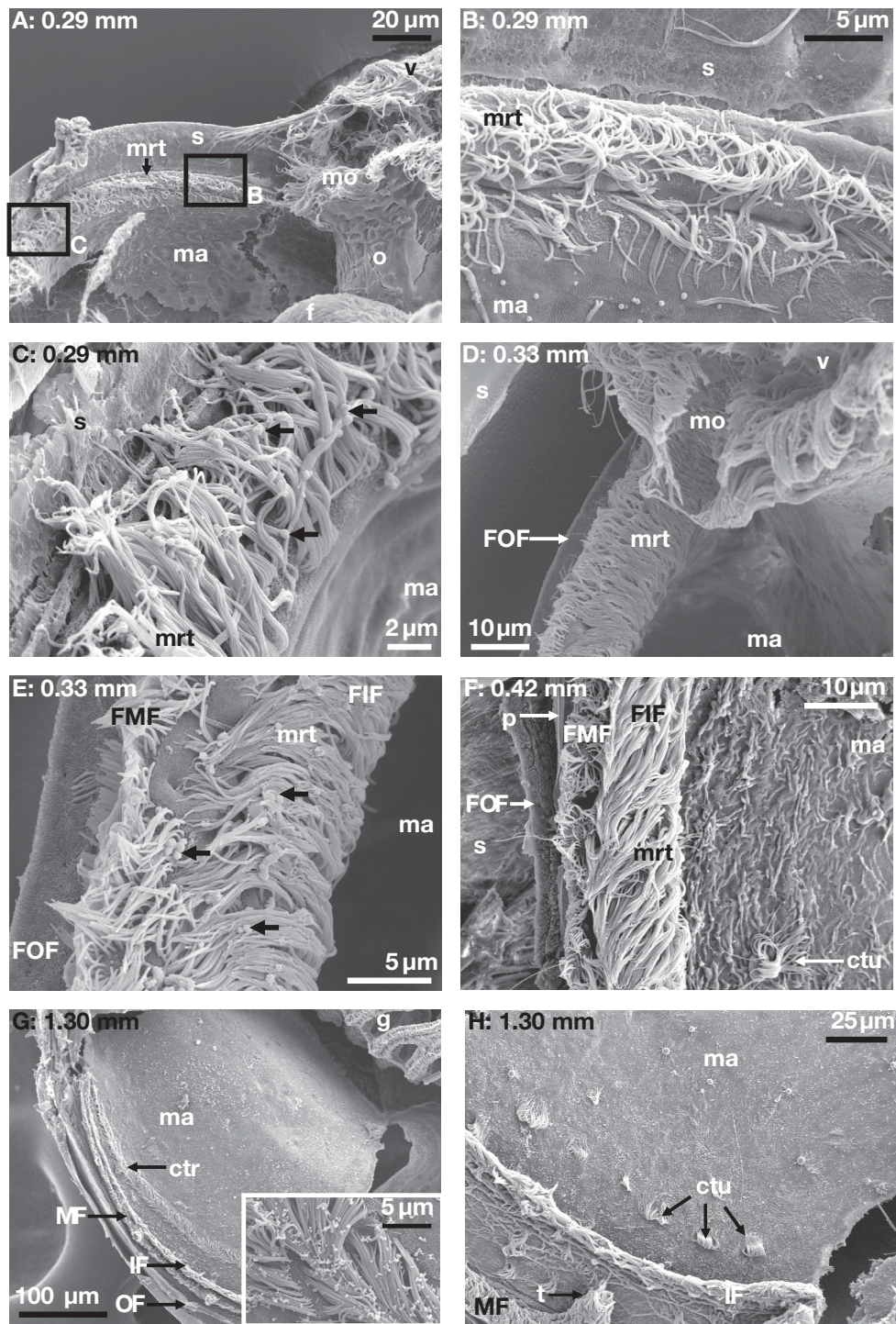


Fig. 1. *Crassostrea gigas*. Larval marginal rejection tract (lateral view). (A) Pediveliger larva (0.29 mm). Boxes B and C: regions shown in detail in the corresponding micrographs. (B) Detail of (A), part of the ciliary tract close to the mouth. (C) Detail of the ciliary tract presented in (A). Arrows: mucus balls. (D) Pediveliger larva (0.33 mm). (E) Detail of the ciliary tract presented in (D). Arrows: mucus balls. (F) Ventral margin of the mantle surface of a 0.42 mm postlarva. (G) Ventral margin of the mantle surface of a 1.30 mm postlarva, showing the new ephemeral marginal tract. Inset: detail of the ciliary tract. (H) Ventral margin of the mantle surface of a 1.30 mm postlarva. ctr: ciliated tract; ctu: ciliary tuft; f: foot; FIF: future inner fold; FMF: future middle fold; FOF: future outer fold; g: gill; IF: inner fold (differentiating); ma: mantle; MF: middle fold (with tentacles); mo: mouth; mrt: marginal rejection tract; o: oesophagus; OF: outer fold; p: periostracum (partially removed from the periostracal groove); s: shell (covered by the periostracum); t: tentacle; v: velum

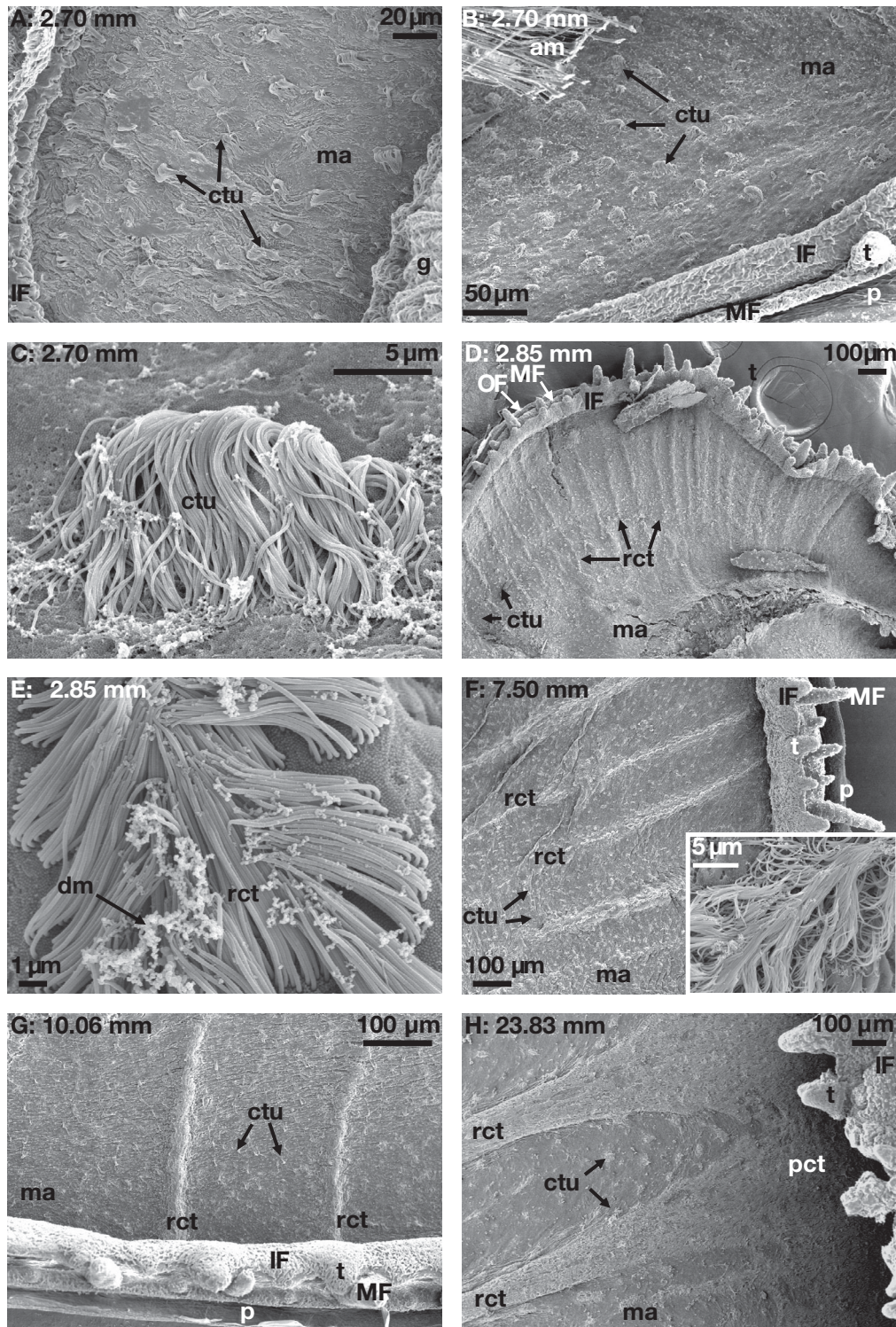


Fig. 2. *Crassostrea gigas*. Transitional ciliation and radial rejection tracts (lateral view). (A) Mantle infrabranchial surface of a 2.70 mm postlarva. (B) Mantle suprabranchial surface of a 2.70 mm postlarva. (C) Detail of a ciliary tuft observed on the mantle surface of a 2.70 mm postlarva. (D) Mantle infrabranchial surface of a 2.85 mm juvenile. (E) Detail of a radial ciliary tract presented in (D). (F) Mantle infrabranchial surface of a 7.50 mm juvenile. Inset: detail of a ciliary tract. (G) Mantle ventral edge of a 10 mm juvenile. (H) Mantle ventral edge of a 24 mm juvenile. am: adductor muscle (sectioned); ctu: ciliary tufts; dm: dehydrated mucus masses; g: gill; IF: inner fold; ma: mantle; MF: middle fold; OF: outer fold; p: periostracum; pct: posterior collecting tract; rct: radial ciliary tract; t: tentacle

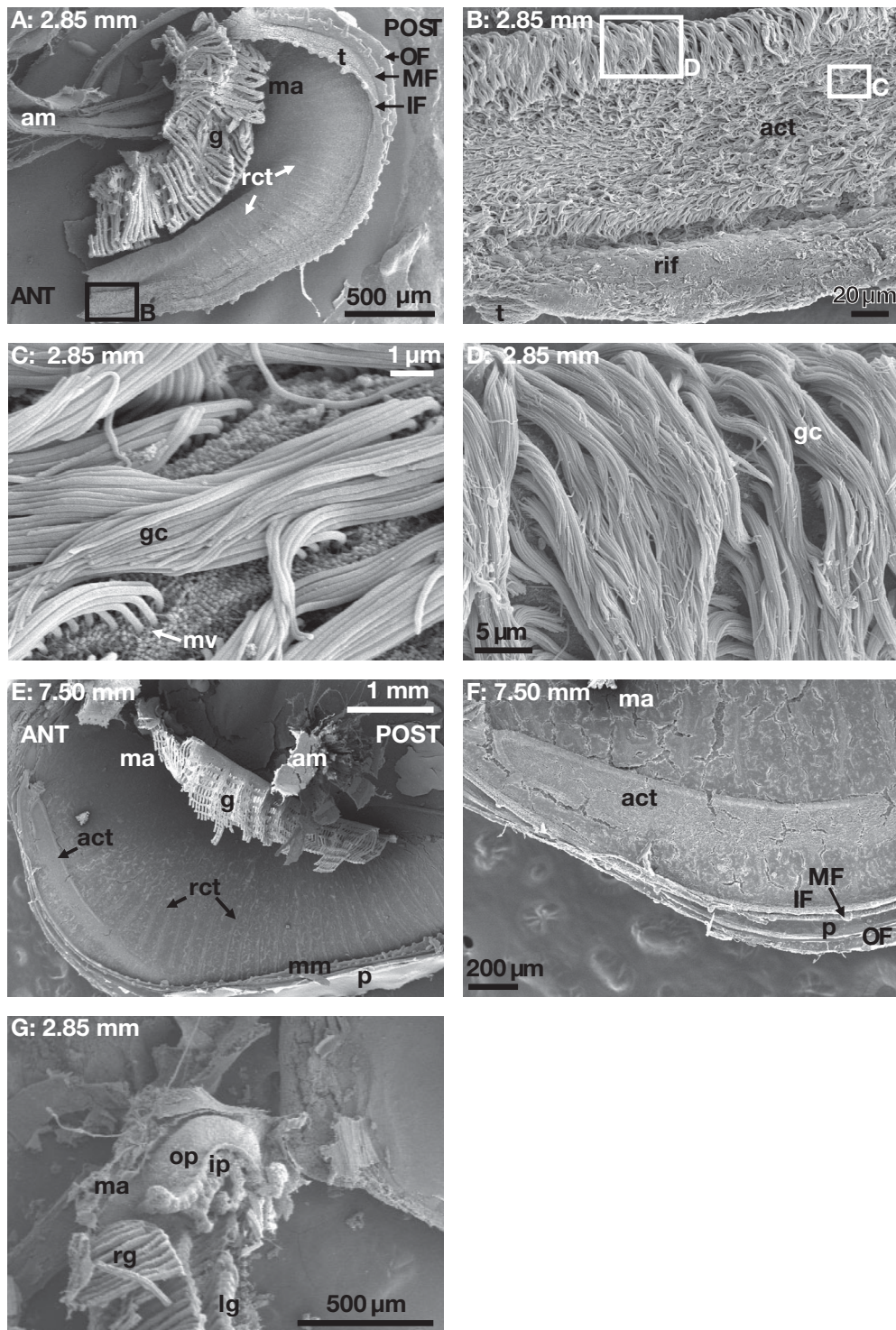


Fig. 3. *Crassostrea gigas*. Juvenile anterior marginal collecting tract. (A) Lateral view of the mantle infrabranchial surface of a 2.85 mm juvenile. Box B: region of detail in corresponding micrograph. (B) Detail of (A). Boxes C and D: regions of detail in corresponding micrographs. (C) Detail of (B). (D) Detail of (B). (E) Lateral view of the mantle infrabranchial surface of a 7.50 mm juvenile; labial palps removed, gill partially removed. (F) Detail of the anterior marginal ciliary tract shown in (E). (G) Ventral view of the labial palps of a 2.85 mm juvenile. act: anterior collecting tract; am: adductor muscle (sectioned); g: gill; gc: grouped cilia; IF: inner fold; MF: middle fold; mm: mantle margin; mv: microvilli; OF: outer fold; op: outer palp; ip: inner palp; lg: left gill; rg: right gill; rif: reduced inner fold; t: tentacle; ANT: anterior; POST: posterior (organ orientation)

ciliary tract, were observed, while the remaining mantle surface remained unciliated (Fig. 1F).

Stage 2. Postlarvae: larval marginal mantle tract evolution to inner mantle fold, appearance of a new marginal ciliary tract

At the 1.30 mm postlarval stage, half (4 of 8) of the specimens observed displayed a new mantle ciliary tract, distinctly different from the previous larval mantle ciliary tract: 15 μm in width, and composed of simple cilia (~8 to 11 μm) beating posteriorly (Fig. 1G). In the remaining individuals, only widely spaced tufts of ~10 to 15 μm simple cilia were observed in the place of this new ventral marginal tract (Figs. 1H & 4B).

The 3-fold condition of the mantle edge was first observed in 1.30 mm postlarvae, the future inner fold region of the larvae becoming the definitive inner fold, and the future middle fold of the larvae becoming the definitive middle fold (Fig. 1G). At this stage, tentacles could be observed on the middle fold, while the developing inner fold retained the abundant ciliation of the larval marginal tract (Figs. 1G,H & 4B).

Stage 3. Transitional phase

No trace of the early postlarval marginal ciliary tract, described above, remained in 2.70 mm postlarvae (Fig. 2A). Both infrabranchial and suprabranchial mantle surfaces presented sparse (spacing ~25 to 50 μm) tufts of simple cilia (~10 to 15 μm) (Figs. 2A–C & 4C).

Stage 4. Juveniles: radial and collecting rejection tracts

The typical oyster radial mantle rejection tracts (Beninger & Veniot 1999) were first observed in 2.85 mm juveniles. Each radial tract, situated atop a ridge, was ~15 μm in width, and the inter-tract distances were ~80 to 130 μm at the distal extremities (Fig. 2D). Radial tract ciliation was composed of apparently simple, ventrally beating cilia (~7 to 8 μm) (Fig. 2E). Non-ordered tufts of simple cilia (~7 to 13 μm) were observed in the inter-ridge spaces.

A marked antero-posterior differentiation of the inner mantle fold became evident, beginning with 2.85 mm individuals: the fold and associated tentacles became smaller toward the ante-

rior extremity (~90 to 115 vs. ~30 μm in width, Fig. 3A).

In 7.50 mm juveniles, the inter-tract distance increased to ~160 to 280 μm (distal extremities). Radial tract width remained constant almost to the distal extremity (Fig. 2F), where they enlarged up to 90 μm in some specimens. Ventrally beating grouped cilia (~8 to 14 μm) were observed on each radial tract. Inter-ridge spaces were punctuated by the previously described ciliary tufts (Fig. 2F).

Radial tract widths and cilia lengths remained constant in 10 mm juveniles, while inter-ridge spaces, still showing ciliary tufts, measured ~180 to 340 μm on the mantle ventral margin (Fig. 2G). Radial rejection tracts were much more developed in 24 mm juveniles, measuring ~80 to 120 μm in width in the proximal (dorsal) portions and enlarging up to ~350 to 470 μm in the distal (ventral) portions, where they fused and created the posterior marginal collecting tract (Figs. 2H & 4D).

The abundantly ciliated anterior marginal collecting tract was first clearly visible in 2.85 mm juveniles (Fig. 3A). It extended from the buccal region to the anterior third of the mantle circumference, and measured ~90 to 130 μm in width (Figs. 3B & 4D). The tract appeared to be comprised of 2 parallel sub-tracts, with respect to the cilia beat orientations: a dorsal band (~20 to 40 μm wide) of dense, apparently grouped, ventrally beating cilia (~15 to 20 μm in length; Figs. 3D & 4D), and a more distal (ventral) band of apparently grouped, anteriorly beating cilia (~10 to 15 μm in length; Figs. 3C & 4D). This anterior marginal collecting tract persisted in larger specimens

Table 2. *Crassostrea gigas*. Mean shell size, age, and critical gill and mantle developmental events associated with larval, postlarval, and juvenile stages. Gill developmental data from Cannuel & Beninger (2006)

| Mean shell size (mm) | Age | Critical events related to gill development | Critical events related to mantle development |
|----------------------|-------|--|--|
| 0.33 | 20 d | Shift from velar to gill particle capture | |
| 0.42 | 22 d | | |
| 1.30 | 29 d | | Re-organization of mantle rejection system Ephemeral marginal tract 'Tractless' mantle stage |
| 2.70 | 36 d | Rapid development of outer demibranchs (metabolic cost) Differentiation of latero-frontal cirri | |
| 2.85 | 10 wk | | Partial radial mantle rejection system functional capability |
| 7.50 | 13 wk | Beginning of principal filament differentiation and gill plication | |
| 10.06 | 16 wk | Near-complete gill plication and fusion | Full radial mantle rejection system functional capability |
| 23.83 | 22 wk | | |

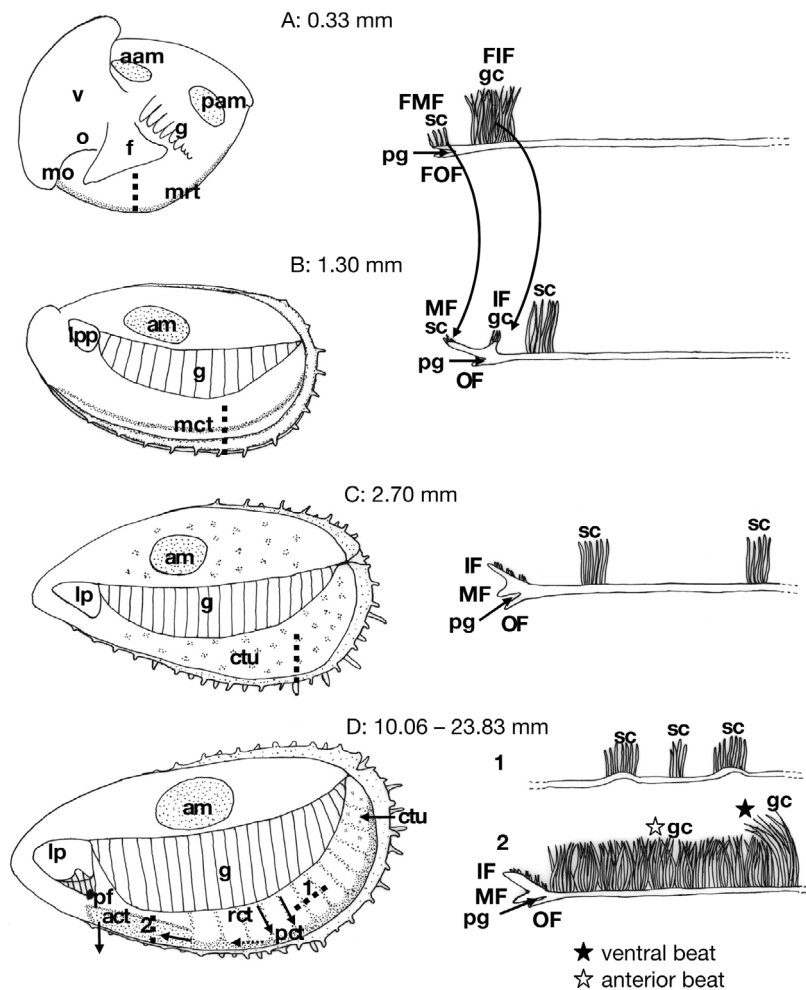


Fig. 4. *Crassostrea gigas*. Summary diagram of mantle ciliation throughout development. Left column: lateral view of whole individual, left valve removed; right column, section viewed perpendicular to plane, indicated by dotted lines at left. (A) Pediveliger larva (0.33 mm) and ciliary types observed in the plane of section (dotted line). (B) Postlarva (1.30 mm) and ciliary types observed in the plane of section (dotted line). (C) Postlarva (2.70 mm) and ciliary types observed in the plane of section (dotted line). (D) Late juvenile (10 to 24 mm) and ciliary types observed in the planes of section 1 and 2 (dotted lines), and direction or probable direction of particle transport on the mantle surface (arrows and dotted arrow respectively). aam: anterior adductor muscle; act: anterior collecting tract; am: adductor muscle; ctu: ciliary tufts; f: foot; FIF: future inner fold; FMF: future middle fold; FOF: future outer fold; g: gill; gc: grouped cilia; IF: inner fold; lp: labial palps; lpp: labial palp primordia; mct: marginal ciliary tract; MF: middle fold; mo: mouth; mrt: marginal rejection tract; o: oesophagus; OF: outer fold; pam: posterior adductor muscle; pct: posterior collecting tract; pf: pseudofeces; pg: periostracal groove; rct: radial ciliary tracts; sc: simple cilia; v: velum

(7.50 mm juveniles; Fig. 3E,F), exhibiting the ciliary types described above.

The labial palps were well developed and markedly ridged at the 2.85 mm stage (Fig. 3G). Their size and location just dorsal to the anterior marginal collecting tract would allow ready deposition of palp pseudofeces on the anterior marginal collecting tract (Fig. 4D).

DISCUSSION

Larval marginal tract

A larval mantle rejection tract, termed the 'mantle rejection tract', has been reported to extend from the buccal region to the posterior extremity in *Mytilus edulis* larvae, although no supporting micrographs have been presented (Bayne 1971). Similarly, Carriker (2001) described (without micrographs) such a tract in *Mercenaria mercenaria* larvae. The presence of a larval oyster mantle marginal rejection tract has heretofore either not been reported or explicitly refuted (Yonge 1926). The first report of a marginal ciliated band in larval oysters was that of Cranfield (1974), later confirmed by Waller (1981), in *Ostrea edulis*; the possible connection to the process of pseudofeces rejection was not explored in these studies. The ventralward beat of the component cilia, observed in the present study, would prevent particles rejected in the buccal region from re-entry into the pallial cavity. The observed rejection of pseudofeces from the posterior extremity of swimming larvae (Yonge 1926) supports this interpretation.

The larval mantle marginal rejection tract described herein for *Crassostrea gigas* resembles that reported in a variety of adult bivalve species, with respect to location and cilia type (Beninger et al. 1999); the evolutionary significance of this point is dealt with below.

Shift to adult mantle rejection system

The adult oyster mantle rejection system is, to date, unique among the Bivalvia studied, due to the presence of multiple radial tracts (Beninger & Veniot 1999) feeding into the marginal collecting tracts. The existence of radial tracts has been known for some time (Yonge 1926, Elsey 1935, Eble & Scro 1996), and the micro-anatomical details have recently been reported (Beninger & Veniot 1999). The present study documents the shift from the single larval-postlarval marginal rejection tract, through the ephemeral secondary marginal ciliated tract when the larval tract is subsumed in the formation of the juvenile

and adult mantle inner fold, penultimately through the tractless stage, to the definitive radial system (Table 2). It is not likely that the ephemeral secondary marginal tract is functional, as at this stage (1.30 mm) the labial palps are not yet functional.

It is thus evident that several re-organizations of the mantle rejection system take place prior to the adoption of the adult system. The final re-organization also corresponds to a reversal of direction of pseudofeces transport along the mantle margin, as pseudofeces in the juvenile and adult are ejected from the anterior region of the mantle. The re-organization to a radial system may correspond to the monomyarian condition, which establishes a single adductor muscle near the centre of the organism.

Although the radial tracts appear relatively soon, at 2.85 mm, the contiguous adult marginal tracts develop successively: the anterior collecting tract at 2.85 mm, and the posterior tract at a size between 10 and 24 mm. Simultaneously, the mantle folds decrease in size in the anterior region of the oyster, facilitating rejection of pseudofeces from the anterior marginal tract. The complete mantle rejection system is thus not functional before 10 to 24 mm (Table 2).

Evolutionary implications

The overwhelming majority of bivalve species studied to date possess a single mantle rejection tract, running antero-posteriorly at various distances from the margin (see Beninger et al. 1999, Beninger & Veniot 1999 for details and references), probably derived from the larval marginal rejection tract described above, as affirmed for *Mercenaria mercenaria* (Carriker 2001). The Pectinidae have no rejection tract, in keeping with the valve-clapping method of pseudofeces expulsion and the lack of a gill ventral particle groove (Beninger et al. 1999). Oysters present several peculiarities in their pallial organs, notably the presence of a heterorhabdic gill in conjunction with grouped latero-frontal cirri and a ventral particle groove. The radial mantle rejection tracts have only been reported in the Ostreidae, and may assist in the regulation of ingestion volume by allowing the removal of material from the ventral particle grooves when the stomach is full (Beninger & Veniot 1999). The observations of the present study show that the single larval-postlarval marginal rejection tract does not persist in the adult, but rather is completely re-organized, passing through a secondary marginal tract stage and then a tractless stage. The final oyster rejection system includes the radial tracts and a new marginal tract differentiated into anterior and posterior components. This suggests that the definitive system is an evolutionarily new

character which is structurally unrelated to the more primitive rejection systems. As noted above, the definitive re-organization corresponds to the reversal of direction of pseudofeces transport on the mantle margin; this is a functional and evolutionary peculiarity of the Ostreidae. These characteristics further separate the Ostreidae not only from the homorhabdic, dimyarian bivalves, but also from the other heterorhabdic monomyarian bivalves.

Ecological and aquacultural implications

To date, we have identified 2 potentially critical stages of gill development in *Crassostrea gigas* in addition to the shift from velar to gill particle capture at metamorphosis: the transition from a V- to a W-shaped gill at 2.70 mm, and the shift from a flat homorhabdic to a deeply plicate heterorhabdic gill beginning at 7.50 mm (Cannuel & Beninger 2006; Table 2, present study). In the present study, we identify another event which severely compromises particle processing ability: the re-organization of the mantle rejection system, beginning at 1.30 mm (Table 2). As this takes place during the V-stage of the gill, and prior to functional capability of the labial palps, it may be concluded that no element of the particle processing system functions efficiently at this stage. In rearing systems where young oysters are fed abundantly and enjoy controlled environmental conditions, this may not be problematic except in the case of unexpected perturbations, such as disease or parasitism. In wild populations, however, this may increase mortality of recruiting oysters under more difficult environmental conditions (Barillé et al. 1997, Cheney et al. 2000, Soletchnik et al. 2005). With respect to the rejection tracts, in particular, particle processing in high turbidities may be problematic during the re-organization of the mantle rejection system, which lasted from 1.30 mm to juveniles at a size between 10 and 24 mm (Table 2).

Full structural, and presumably functional, capability of the *Crassostrea gigas* mantle rejection system occurs at a size between 10 and 24 mm, corresponding to the size range at which the heterorhabdic gills achieve significant plication (Cannuel & Beninger 2006; Table 2, present study). As the deep oyster gill plication is responsible for the separation of initial acceptance and rejection tracts on the gill (Beninger et al. 2005), the timing of these 2 developmental events results in simultaneously, fully functional gills and mantle in individuals over the range 10 to 24 mm (Table 2). Since the labial palps are structurally functional at 2.85 mm, oysters which have attained full gill and mantle functional capability therefore also possess

full labial palp functional capability. Particle processing should hence be facilitated in *C. gigas*, beginning at a size between 10 and 24 mm. This may be compared with full structural, and presumably functional, capability at ~7 mm in *Placopecten magellanicus* (Veniot et al. 2003), a difference all the more remarkable given the significantly higher rearing temperature for *C. gigas* (24 to 25°C vs. 14°C for *P. magellanicus*). The extended developmental time for *C. gigas* reflects its more complex gill development, which produces extensive organic gill junctions after initiation of heterorhabdic differentiation (Cannuel & Beninger 2006).

The developmental events described above should improve our understanding of basic biological aspects likely to influence growth and mortality in post-metamorphic *Crassostrea gigas*. Physiological experimentation at the identified stages, while difficult, may provide quantitative information on how pallial organ development influences particle processing in these early life stages.

Acknowledgements. We thank J. C. Cochard, S. Pouvreau, C. Mingant and the Ecloserie expérimentale d'Argenton (IFREMER) for facilities, and for help with larval and postlarval rearings. In particular, we thank one of the anonymous reviewers for pertinent remarks on a previous version of this manuscript. We are grateful to S. Angeri (Vendée Naissain) for providing the juvenile specimens, and A. Barreau for assistance in scanning electron microscopy. Research funding was provided by the Région des Pays de Loire (PhD stipend to R.C.), and partial operational funding was provided by IFREMER (Contrat universitaire IFREMER/ EMI No. 03-2-2521559).

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Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

*Submitted: June 23, 2005; Accepted: March 29, 2006
Proofs received from author(s): September 25, 2006*