ELSEVIER

Tissue and Cell 37 (2005) 153–165

Tissue&Cell

www.elsevier.com/locate/tice

Branchial chamber tissues in two caridean shrimps: the epibenthic *Palaemon adspersus* and the deep-sea hydrothermal *Rimicaris exoculata*

Anne-Sophie Martinez [a], Guy Charmantier [b], Philippe Compère [c], Mireille Charmantier-Daures [b,*]

[a] *LBBM, Université de Caen, Esplanade de la Paix, 14032 Caen cedex, France*
[b] *Equipe Adaptation Ecophysiologique et Ontogenèse, UMR 5171 Génome, Populations, Interactions, Adaptation, Université Montpellier II, Cc 092, Place E. Bataillon, 34095 Montpellier cedex 05, France*
[c] *Laboratoire de Biologie Générale et de Morphologie Ultrastructurale, Institut de Zoologie, Université de Liège, 22 quai Edouard Van Beneden, B-4020 Liège, Belgique*

Received 27 August 2004; received in revised form 13 December 2004; accepted 22 December 2004

Abstract

The structure of the epithelia of the branchial chamber organs (gills, branchiostegites, epipodites) and the localization of the Na^+,K^+-ATPase were investigated in two caridean shrimps, the epibenthic *Palaemon adspersus* and the deep-sea hydrothermal *Rimicaris exoculata*. The general organization of the phyllobranchiate gills, branchiostegites and epipodites is similar in *P. adspersus* and in *R. exoculata*. The gill filaments are formed by a single axial epithelium made of H-shaped cells with thin lateral expansions and a basal lamina limiting hemolymph lacunae. In *P. adspersus*, numerous ionocytes are present in the epipodites and in the inner-side of the branchiostegites; immunofluorescence reveals their high content in Na^+,K^+-ATPase. In *R. exoculata*, typical ionocytes displaying a strong Na^+,K^+-ATPase specific fluorescence are observed in the epipodites only. While the epipodites and the branchiostegites appear as the main site of osmoregulation in *P. adspersus*, only the epipodites might be involved in ion exchanges in *R. exoculata*. In both species, the gill filaments are mainly devoted to respiration.

Keywords: Osmoregulation; Ionocyte; Gills; Na^+,K^+-ATPase; *Palaemon*; *Rimicaris*

1. Introduction

Osmoregulation is one of the most important adaptive physiological processes permitting the successful establishment of a species in a given habitat (Charmantier, 1998). In marine Crustaceans, this mechanism is based on active ion transport performed by highly differentiated osmoregulatory epithelia of the branchial chambers hosting specialized cells or ionocytes (Taylor and Taylor, 1992).

Although a large amount of data exists on the pattern of osmo- and/or ionoregulation in different species of caridean shrimps (review in Mantel and Farmer, 1983), the structure of the ionoregulating tissues of the Caridea is poorly documented (see review in Martinez, 2001). The available data are limited to the gills in *Palaemonetes varians* (Allen, 1892), *Crangon vulgaris* (Debaisieux, 1970), *Macrobrachium olfersii* (Freire and McNamara, 1995; McNamara and Lima, 1997) and to the epipodites in *Crangon vulgaris* (Debaisieux, 1970). No information is available on the branchiostegites, which are known as osmoregulatory structures in decapod species such as the thalassinid shrimp *Calianassa jamaicense* (Felder et al., 1986), the peneid shrimps *Penaeus aztecus* (Talbot et al., 1972) and *P. japonicus* (Bouaricha et al., 1994) and the lobster H*omarus gammarus* (Haond et al., 1998; Lignot et al., 1999; Lignot and Charmantier, 2001). In addition there is no information on the structure of the branchial chamber tissues in the shrimps that live around deep hydrothermal vents.

R. exoculata (Williams and Rona, 1986), family Alvinocarididae (Christoffersen, 1986) is the dominant species among the fauna of the Mid-Atlantic Ridge

* Corresponding author. Tel.: +334 6714 3675; fax: +334 6714 9390.
E-mail address: charmantier.daures@univ-montp2.fr (M. Charmantier-Daures).

0040-8166/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tice.2004.12.004

hydrothermal vent sites (Galkin and Moskalev, 1990; Gebruk et al., 1997; Tunnicliffe et al., 1998; Van Dover, 2000). This species forms exceptionally dense clusters (up to 3000 individuals per m^2) on sulfide mounds associated with active venting (Segonzac et al., 1993; Van Dover, 1995). The adult shrimps live permanently in or very close to variable and extreme environmental conditions such as high temperature, high sulfide and metal content, high level of carbon dioxide, low oxygen level, and low pH (Truchot and Lallier, 1998; Sarradin et al., 1998a; Sarradin et al., 1998b; Sarradin et al., 1998a,b, 1999). Temperatures appear to range between 10 and 30 °C with an upper limit at 70 °C (Segonzac et al., 1993; Lakin et al., 1997; Lallier and Truchot, 1997; Truchot and Lallier, 1998). From the scarce and controversial available information, salinity of the hydrothermal fluid seems variable from 2.8 to 68% depending to the sites (Chevaldonné, 1997; Truchot and Lallier, 1998; Van Dover, 2000).

The objective of the study was to compare the structure of the organs of the branchial chambers in two caridean shrimps living in demanding but different habitats: *Palaemon adspersus*, an epibenthic shrimp which lives in coastal lagoons where salinity is very variable, able to strongly hyper-hyporegulate (Martinez, 2001) and the deep-sea hydrothermal *R. exoculata*. The presence of typical cells involved in ion exchanges or ionocytes was investigated through light and electron microscopy; immunocytochemistry was used to localize Na^+,K^+-ATPase, one of the main enzyme involved in active ion transport.

2. Materials and methods

2.1. Animals

Adults of *Palaemon adspersus* were caught in the Mauguio lagoon (Hérault, France). The shrimps were maintained at the Montpellier laboratory for ca. two weeks in 3000 l tanks containing aerated and re-circulated natural seawater (37 ± 1% at 20 ± 1 °C) under a 12 h L/12 h D photoperiod. They were fed three times a week with defrosted cooked mussels. The shrimp mean cephalothoracic length was 10.14 ± 0.86 mm.

Adults of *R. exoculata* were collected by French and American submarines at a depth of about 3000 m, on the Rainbow and Logatchev sites of the Mid-Atlantic Ridge [36°11′N–33°57′W and 14°45′N–44°58′W (Tunnicliffe et al., 1998; Van Dover, 2000)], respectively, during the MARVEL (1997), and ATOS and DIVERSExpedition (2001) missions. The shrimp cephalothoracic length was 10–25 mm. Only a small number of shrimps was available. The hydrothermal shrimps, which seem incapable of long-term survival outside the high-pressure environment of the deep-sea, were dissected and fixed as soon as they reached the boat. For both species, the observations were conducted in stage C specimens selected after microscopic examination of the tip of a pleopod according to the method of Drach and Tchernigovtzeff (1967).

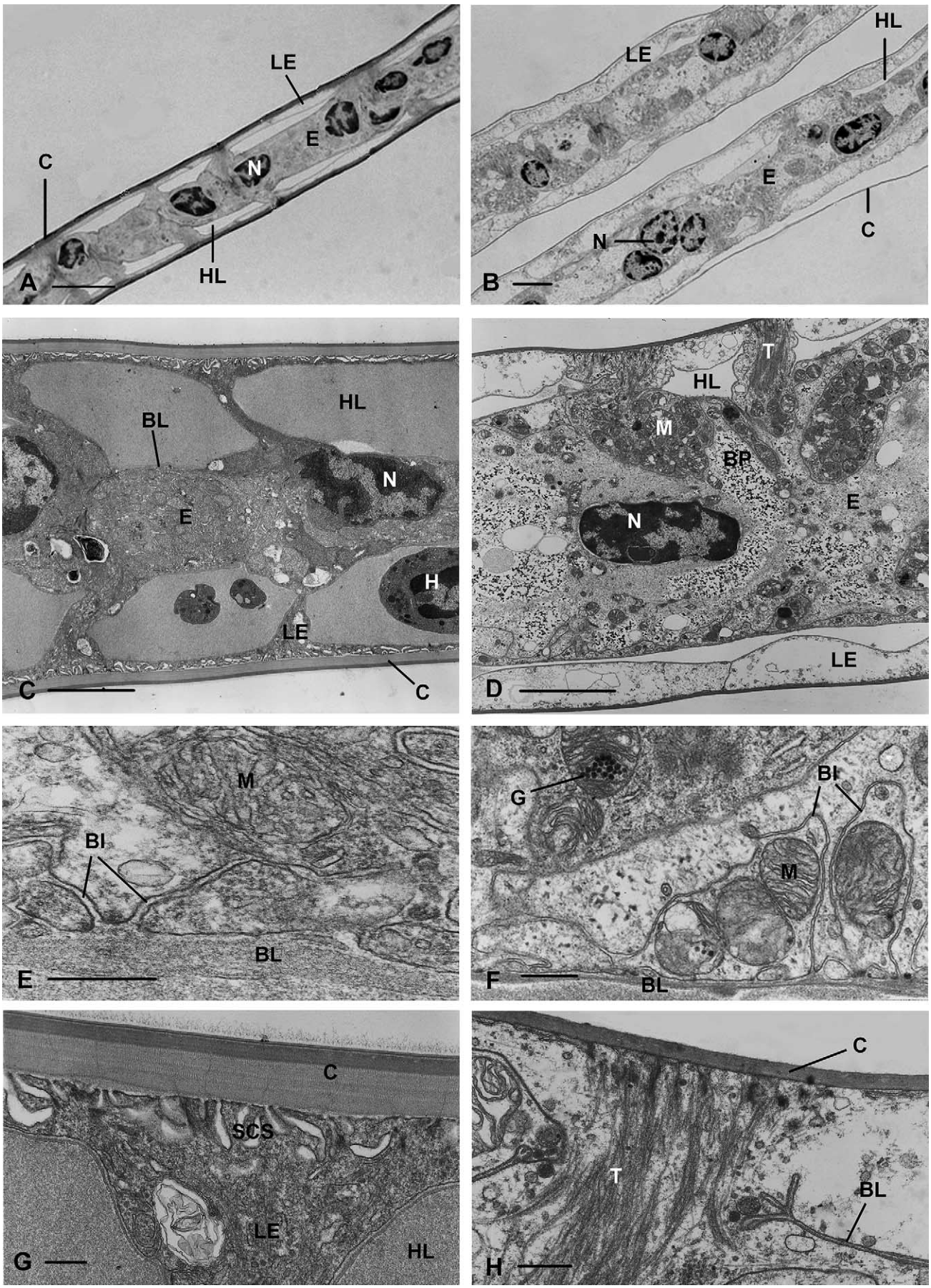
2.2. Light microscopy

The cephalothorax from freshly killed individuals (by section of the cerebroid ganglia) was longitudinally cut into two halves which were fixed for 24 h in Bouin's fixative. This fixation was performed on board for *R. exoculata*. The specimens were then fully dehydrated in a graded ethanol series and embedded in paraplast. Sections (5 μm) were cut on a Leitz Wetzlar microtome, collected on albumine-glycerine slides and stained with Masson Trichrome (variant Goldner) (Martoja and Martoja-Pierson, 1967).

Immunostaining localization of Na^+,K^+-ATPase was performed with a mouse monoclonal antibody IgGα5 raised against the α-subunit of the chicken Na^+,K^+-ATPase (Takeyasu et al., 1988). This antiserum, previously used on *Porcellio scaber* (Ziegler, 1997), *Carcinus maenas* (Lucu and Flik, 1999), *Astacus leptodactylus* (Barradas et al., 1999b) and *Homarus gammarus* (Lignot et al., 1999; Lignot and Charmantier, 2001) was kindly provided by D.M. Fambrough (Baltimore, Md., USA).

Transverse sections of the tissues (3 μm) were collected on poly-L-lysine-coated slides. The sections were pre-incubated for 10 min in 0.01 mM Tween 20, 150 mM NaCl in 10 mM phosphate buffer saline (PBS), pH 7.3, treated with 50 mM NH_4Cl in 20 mM phosphate buffer saline (PBS), pH 7.3 for 5 min, then incubated for 10 min with a blocking solution (BS) containing 1% bovine serum albumine (BSA) and 0.1% gelatin in 20 mM PBS, pH 7.3. Droplets of the primary antibody (dilution: 20 $\mu g \cdot ml^{-1}$) were placed on the sections that were incubated for 2 h in a wet chamber at room temperature. Control sections were incubated without primary antibody. After being washed in BS, the sections were incubated for 1 h in droplets of the secondary antibody, fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (H&L; Jackson Immunoresearch, West Baltimore, Md.). The

Fig. 1. Gill lamellae of *Palaemon adspersus* (A, C, E, G) and *Rimicaris exoculata* (B, D, F, H); semi-thin sections (A, B) and transmission electron micrographs (C–H). A, B: The epithelium of the lamellae is made of horizontally disposed H-shaped cells with sub-cuticular extensions. C, D: Lamellar epithelium cells; note the difference in size of the hemolymph lacunae between the two species, and the area containing black particules near the nucleus in *R. exoculata* (D). E–H: Detail of lamellae cells; E: membrane infoldings on the axial zone in *P. adspersus*; F: light system of membranes associated with mitochondria in *R. exoculata*; note the granules in the mitochondria; G: apical part with sub-cuticular spaces in *P. adspersus*; H: zone of insertion of tonofilaments on the cuticle in *R. exoculata*; note the reduced hemolymph lacunae bordered by the basal lamina. Scale bars: (A, B) 10 μm; (C, D) 5 μm; (E–H) 500 nm. BI: Basal infolding; BL: basal lamina; BP: black particules; C: cuticle; E: epithelium; G: granule; H: hemocyte; HL: hemolymph lacuna; LE: lateral expansion; M: mitochondria; N: nucleus; SCS: sub-cuticular spaces; T: tonofilaments.


LE
E
C
N
HL
A
LE
HL
E
N
C
B
HL
BL
N
E
H
LE
C
C
T
HL
M
BP
E
N
LE
D
M
BI
BL
E
G
BI
M
F
BL
C
SCS
LE
HL
G
C
T
BL
H

sections were mounted in 80% glycerine, 20% PBS plus 2% *N*-propyl-gallate to retard photobleaching. They were examined on a fluorescent microscope (Leitz Diaplan coupled to a Ploemopak 1-Lambda lamp) with the appropriate filter set (450–490 nm band-pass excitation filter).

2.3. Transmission electron microscopy (TEM)

The technique of Pottu-Boumendil (1989) was used. Dissected pieces of gills, epipodites and branchiostegites were fixed on ice for 2 h with 2.5% glutaraldehyde in a mixture of seawater and freshwater, pH 7.4, adjusted to the hemolymph osmotic pressure to prevent osmotic shocks. The branchiostegites and epipodites were decalcified for five days in EDTA-Na_2 0.2 M, pH 8. Samples were post-fixed for 2 h at 4 °C in OsO_4, washed in distilled water and dehydrated in a graded ethanol series and propylene oxide, then embedded in Spurr's resin. Semithin and ultrathin sections were cut on a Reichert OMU3 ultramicrotome. The first sections were stained with toluidine blue. Ultrathin sections were contrasted with 2% uranyl acetate in 70° alcohol and lead citrate, and they were observed on a JEOL 1200 EX2 transmission electron microscope at 70 kV.

3. Results

3.1. Gills

The eight pairs of gills of *P. adspersus* are phyllobranchiate gills. The flattened lamellae are attached along the two outer faces of the triangular gill axis, forming two rows oriented at a 40° angle. The branchial lamellae are 20 μm thick (Fig. 1A and C). They are composed of H-shaped epithelial cells with a thick axial zone (6–10 μm) containing a voluminous round or oval central nucleus (5–6 μm). Lateral thin expansions (0.5 μm) extend under the thin cuticle (0.2–0.8 μm) and limit two rows of hemolymph lacunae, where few hemocytes are present. The cytoplasm of the epithelial cells is generally electron-dense, especially around the nuclei (Fig. 1C). The axial zone presents a dense system of membrane infoldings opened on the basal lamina and closely associated with numerous round or oval mitochondria (0.3–1.4 μm) (Fig. 1C). These infoldings are orientated parallel, rather than perpendicular, to the length of the lamellae (Fig. 1E). The lateral expansions of the epithelial cells contain few cytoplasmic organelles. Only sub-cuticular spaces, irregularly distributed, can be observed in the lamellae (Fig. 1C and G).

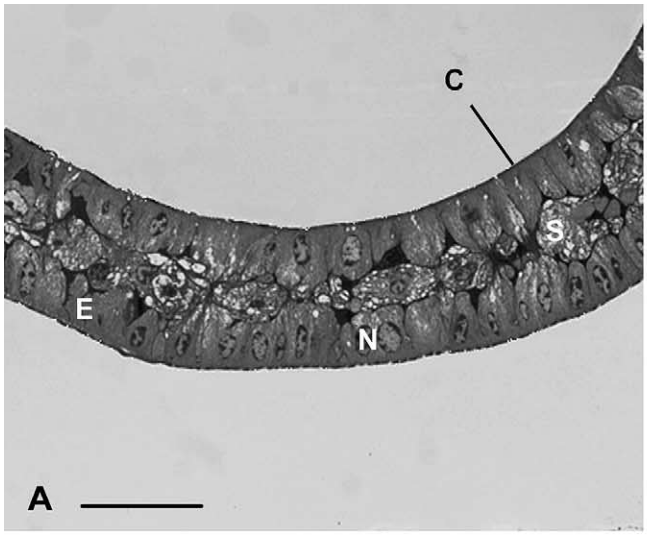
Regarding the immuno-localization of Na^+,K^+-ATPase, classical fixation and paraffin-embedding procedures yielded good antigenicity as observed with the fluorescent micrographs (Fig. 4A–D). Controls showed no specific binding within the epithelia of gills, branchiostegites (Fig. 4A) and epipodites (results not shown). Autofluorescence was observed along the cuticle, mostly at the epicuticle level (Fig. 4D). Na^+,K^+-ATPase specific labelling was detected only in the axial zone of the epithelial cells of the gills; it was absent in their thin lateral expansions (Fig. 4B).

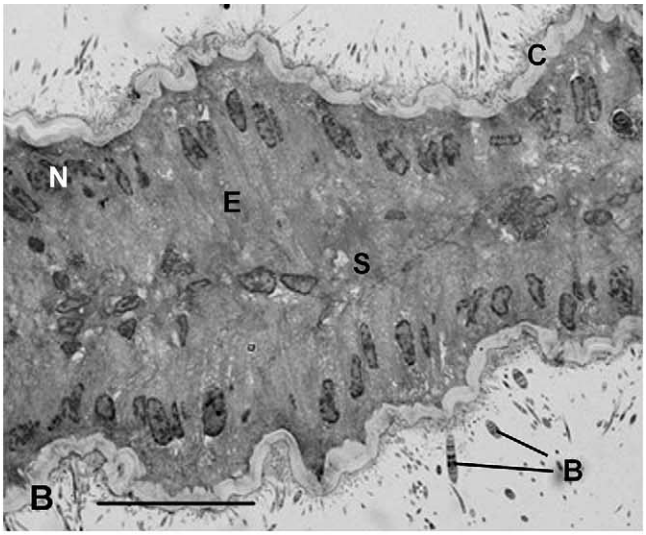
R. exoculata possess 10 pairs of phyllobranchiate gills. The branchial lamellae (15–40 μm thick) show a single layer of H-shaped epithelial cells which almost fully occupy the volume of the lamellae, bordered by a thin cuticle (0.3–0.5 μm) (Fig. 1B and D). The nuclei, round or oval (8–10 μm) are irregularly located in the central and dense perikaryon (13–20 μm thick) of the cells. Lateral cell expansions (2.5 μm thick) limit reduced lateral hemolymph lacunae lined by the basal lamina (Fig. 1B and D). The cytoplasm of the axial part of the epithelial cells contains numerous mitochondria (1 μm) with either well-shaped, regular and dense crests or short and disorganized crests; both are grouped and surrounded by a light system of membranes sometimes in relation with the basal lamina (Fig. 1F). Some mitochondria contain granules of 40 nm in diameter (Fig. 1F). Large vesicules or clear areas containing black particules are frequently located around the nucleus (Fig. 1D). The cytoplasm of the lateral expansions of the epithelial cells hosts few organites except at the pilar junctions where bundles of long tonofilaments coming for the axial part are inserted on the cuticle (Fig. 1H). No Na^+,K^+-ATPase specific labelling was observed in the gills, neither in the axial zone nor in the lateral expansions of the epithelial cells (Fig. 4E).

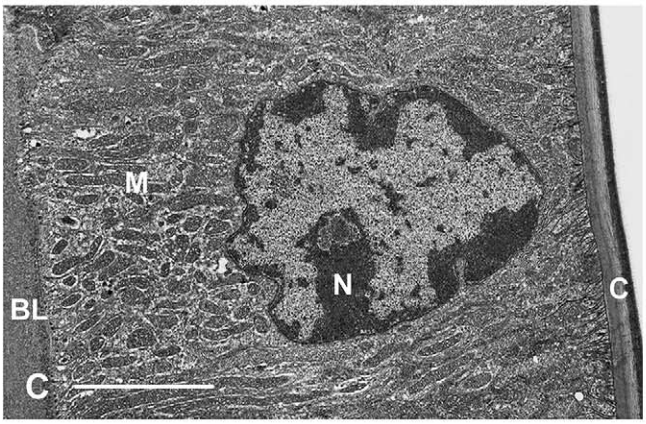
3.2. Epipodites

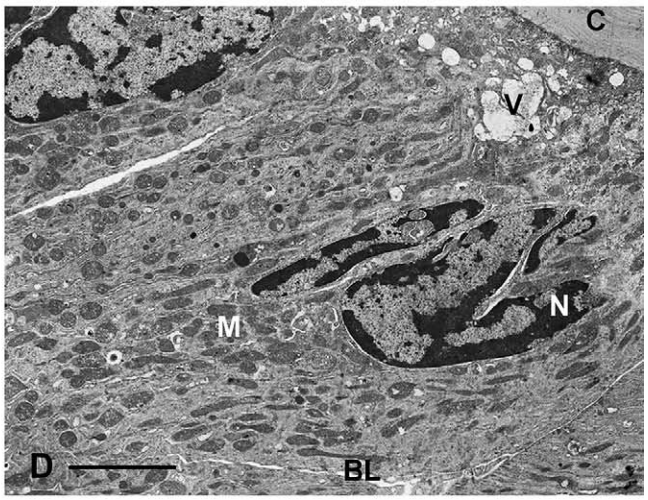
Each branchial chamber of *P. adspersus* contains three epipodites associated with each maxilliped: the first is very small, the second (65 μm thick) has a common base with the podobranch, the third is a well-developed lamellar organ (100 μm thick). Both sides of the epipodites are lined by a thick regular epithelium made up of prismatic cells (20–25 μm high) with voluminous oval nuclei (8 μm in diameter) (Fig. 2A). The central axis of the organs is occupied by a lamellar septum made up of connective tissue and hemolymph lacunae; the basal laminae of the two facing epithelial layers are close in some places. No pillar structures have been observed between them. The cytoplasm of the epithelial cells contains numerous elongated mitochondria

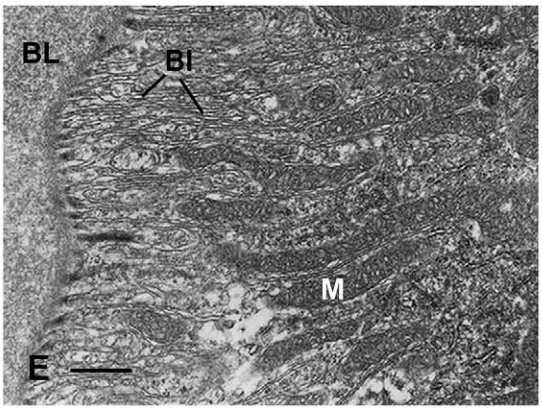
Fig. 2. Epipodites of *Palaemon adspersus* (A, C, E, G) and *Rimicaris exoculata* (B, D, F, H); semi-thin sections (A, B) and transmission electron micrographs (C–H). A, B: General organization of the two facing epithelia. C, D: Epithelial cells. E, F: Basal area with membrane infoldings and mitochondria. G, H: Apical pole with microvilli. Scale bars: (A, B) 50 μm; (C, D) 5 μm; (E–H) 1 μm. AM: Apical microvilli; B: bacteria; BI: basal infolding; BL: basal lamina; C: cuticle; E: epithelium; M: mitochondria; N: nucleus; S: septum; V: vesicle.

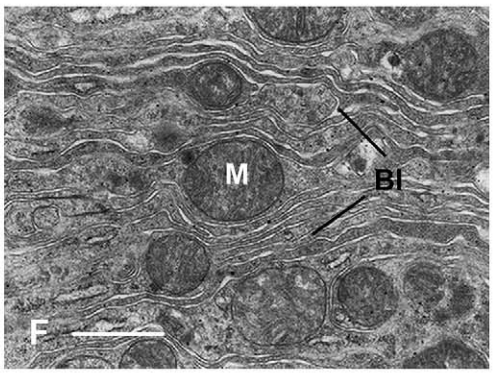

C
S
E
N
A

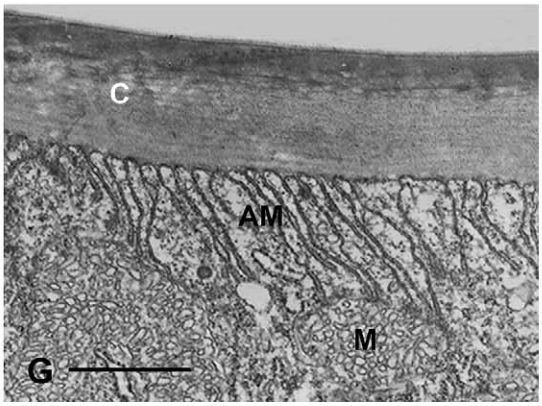

C
N
E
S
B
B

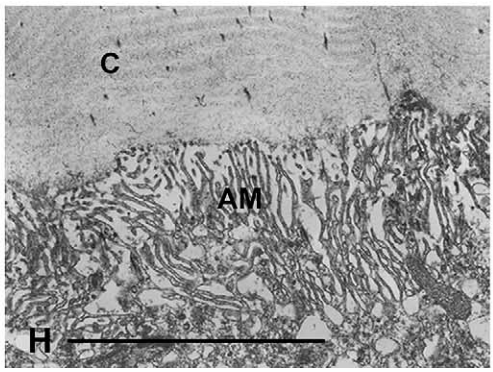

M
N
BL
C
C


C
V
M
N
D
BL


BL
BI
M
E


M
BI
F


C
AM
M
G


C
AM
H

(2.5 μm in average length), closely associated with dense and deep infoldings of the basal cell membrane orientated perpendicular to the surface of the epithelium (Fig. 2C and E). Under the thin cuticle (1 μm), the apical membrane of the cells forms numerous microvilli (1–1.5 μm high), sometimes associated with small mitochondria (Fig. 2G).

A strong Na^+,K^+-ATPase immunolabelling was detected in the two layers of the epithelium of the epipodites, mostly in the basal and median parts of the cells. No immunoreactivity was detected in the axial septum (Fig. 4C).

The three pairs of epipodites of *R. exoculata* are lamellar organs with an irregular thickness (80–160 μm). The first is a well-developed ovoid lamella; the second is rudimentary and foliated; the third is reduced and forms an outgrowth at the base of the third maxilliped. Their general organization is illustrated in Fig. 2B. They are limited by two single and dense epithelial layers made up of high prismatic cells (30–70 μm thick) with big ovoid nuclei (10–20 μm) at the apical side. A thin and dense conjunctive tissue with numerous nuclei and small hemolymph lacunae is present in the axial zone of the epipodites. No pillar cell was observed. Both epithelia are limited by a thick cuticle (5 μm) covered by bacteria. The cytoplasm of the epithelial cells contains numerous mitochondria displaying various shapes (round, oval or elongated) and sizes (3 μm long, 0.3–1.5 μm in diameter) (Fig. 2D). They are closely associated with a dense network of deep basal infoldings. These well-developed infoldings have a variable length according to the cells or cellular areas (Fig. 2D and F). Abundant and irregular apical microvilli (3 μm high) are observed under the cuticle, sometimes associated with small vesicules (Fig. 2D and H). The epipodites showed a strong and regular Na^+,K^+-ATPase fluorescence in the cells of the two epithelial layers. The immunolabelling was absent in the axial septum (Fig. 4F).

3.3. Branchiostegites

The general organization of the branchiostegite of *P. adspersus* is illustrated in Fig. 3A. This structure, approximately 135 μm thick, comprises two irregular thick epithelia maintained by pillar cells which cross a voluminous central hemolymph lacuna. The outer epithelium is made up of high prismatic irregular cells (about 25–40 μm high) under a thick cuticle (40–50 μm) which forms part of the lateral carapace of the cephalothorax. The epithelium lining the inner side of the branchiostegite (Fig. 3C), irregular and thick (20–40 μm high), is covered by a thin cuticle (0.5 μm). In both epithelia, the nuclei are big (10 μm in diameter) and irregularly shaped. The outer epithelium contains mainly clear vesicules of various sizes and no specific differentiation except bundles of tonofilaments (not illustrated). The cells of the inner epithelium contain numerous round or oval mitochondria (0.5–3 μm) orientated perpendicular to the surface of the epithelium. They are closely associated with dense and deep basal cellular infoldings which almost reach the apical side of the cells (Fig. 3E and G). No apical microvilli were observed (Fig. 3G). The inner epithelium of the branchiostegite showed a very strong Na^+,K^+-ATPase fluorescence particularly at the basal side of the cells. The enzyme specific labelling was absent from the central zone of the pillar structures. No fluorescence was detected in the outer epithelium of the branchiostegite (Fig. 4D).

The thick (130 μm) branchiostegite of *R. exoculata* is lined by two irregular epithelial layers partly separated by clusters of a central hemolymph lacuna (Fig. 3B). The outer side of the epithelium (25–40 μm thick) is situated below a thick cuticle (30 μm) forming the lateral carapace of the cephalothorax. The inner epithelium (15–50 μm thick) is covered by a thin cuticle (0.5 μm). The nuclei are ovoid, variable in size (5–15 μm) and irregularly placed. Dense pilar structures join the two cuticles. The cytoplasm of the internal epithelial cells contains numerous small and round mitochondria (0.5–0.8 μm) (Fig. 3D). The basal side of the cells shows short (2.5–4 μm) and scarce basal infoldings (Fig. 3F). Toward the central hemolymph lacuna, some cells present voluminous basal bulges containing vesicules loaded with black particules of different densities (Fig. 3D). They are part of the cell and boarded by the basal membrane of the epithelium. Mitochondria are irregularly located beneath apical microvilli (1 μm high) (Fig. 3H). No Na^+,K^+-ATPase immunoreactivity was detected in the inner and outer epithelia of the branchiostegites (Fig. 4G).

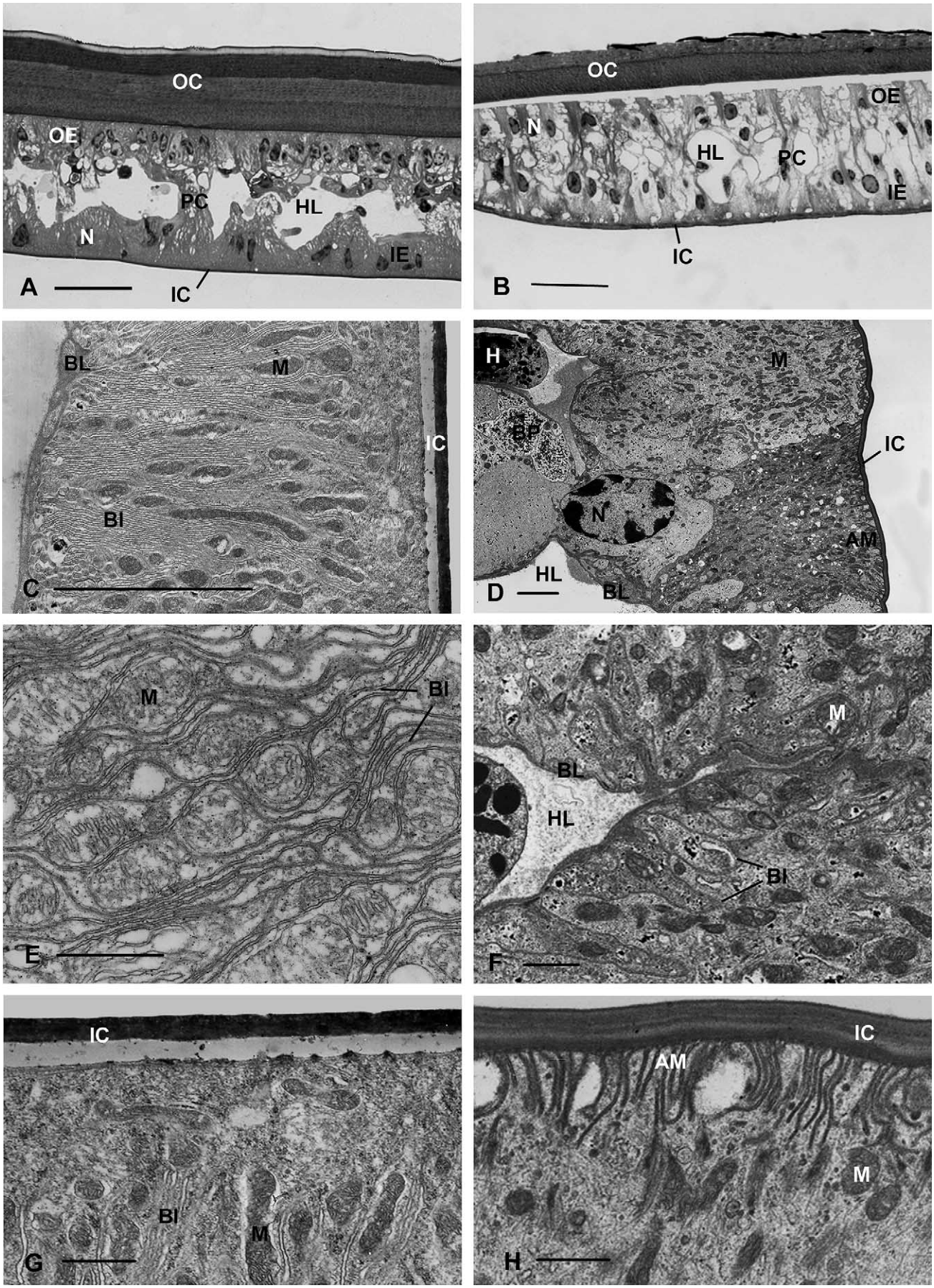
4. Discussion

4.1. Gills

P. adspersus and *R. exoculata* possess phyllobranchiate gills as the other palaemonid shrimps and the brachyuran crabs (Cuénot, 1893; Debaisieux, 1970; Taylor and Taylor, 1992).

The branchial lamellae of *P. adspersus* and *R. exoculata* show an axial epithelium with H-shaped cells. The perikarya of the cells are situated in the longitudinal medial septum while the lateral expansions form thin sheets along the inner surface of the cuticle. The basal lamina of the epithelial

Fig. 3. Branchiostegites of *Palaemon adspersus* (A, C, E, G) and *Rimicaris exoculata* (B, D, F, H); semi-thin sections (A, B) and transmission electron micrographs (C–H). A, B: General organization. C, D: Epithelial cells; note the voluminous basal bulges with black particules in *R. exoculata* (D). E, F: Basal area; note the dense infoldings in *P. adspersus* (E). G, H: Apical part. Scale bars: (A, B) 50 μm; (C, D) 5 μm; (E–H) 1 μm. AM: Apical microvilli; BI: basal infolding; BL: basal lamina; BP: black particules; H: hemocyte; HL: hemolymph lacuna; IC: inner cuticle; IE: inner epithelium; M: mitochondria; N: nucleus; OC: outer cuticle; OE: outer epithelium; PC: pillar cell.


OC
OE
PC
HL
N
IE
IC
A
OC
OE
N
HL
PC
IE
IC
B
BL
M
IC
BI
C
H
M
BP
IC
N
AM
HL
BL
D
M
BI
E
M
BL
HL
BI
F
IC
BI
M
G
IC
AM
M
H

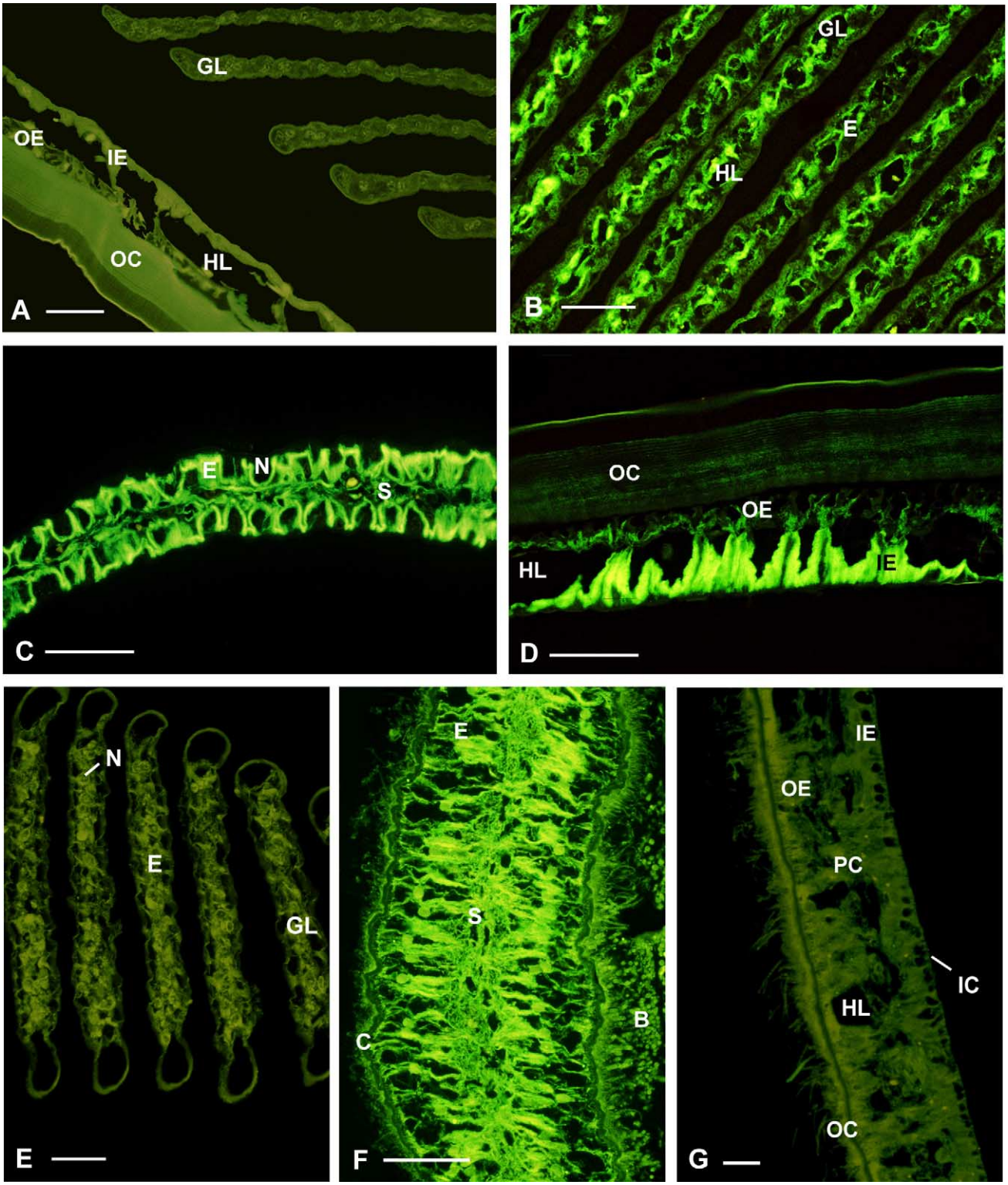


Fig. 4. *P. adspersus* and *R. exoculata*. Immunolocalization of Na^+,K^+-ATPase in the gills, epipodites and branchiostegites. A, B, C, D: *P. adspersus*; A: control branchial chamber including gills and branchiostegite; B: gill lamellae; C: epipodite; D: branchiostegite. E, F, G: *R. exoculata*; E: gill lamellae; F: epipodite; G: branchiostegite. Scale bars: 50 μm. B: Bacteria; Br: branchiostegite; C: cuticle; E: epithelium; GL: gill lamellae; HL: hemolymph lacuna; IC: inner cuticle; IE: inner epithelium; LE: lateral expansion; MC: marginal channel; N: nucleus; OC: outer cuticle; OE: outer epithelium; PC: pillar cell; S: septum.

cells limits lateral hemolymph lacunae. The organization of the branchial lamellae of *R. exoculata* is similar to that observed in *P. adspersus* and in other palaemonid and crangonid shrimps (Papathanassiou and King, 1983; Doughtie and Rao, 1984; Papathanassiou, 1985; Patil and Kaliwal, 1989; Freire and McNamara, 1995). This structure differs from the gill organization of brachyuran crabs in two ways: (i) the epithelium, which is axial in *P. adspersus* and *R. exoculata*, is laterally located in the brachyura (Finol and Croghan, 1983; Compère et al., 1989; Goodman and Cavey, 1990; Farelly and Greenaway, 1992); (ii) the axial conjunctive septum described in most brachyurans as located between the two epithelial layers, seems absent in *P. adspersus* and *R. exoculata*.

In *P. adspersus*, the axial zone of the epithelial cells of the gills possesses a network of basal internal membranes closely associated with numerous mitochondria. Na^+,K^+-ATPase is also slightly present. These cells appear as atypical ionocytes. They differ from typical ionocytes by the low number of membrane infoldings, their irregular orientation and their scarce openings on the basal lamina (reviews in Taylor and Taylor, 1992; Péqueux, 1995), but the presence of the principal enzyme involved in active ion transport is indisputable. These cells are thus probably involved in osmoregulatory active ion pumping. Anatomical evidence has lead to the same hypothesis in other caridean shrimps (Doughtie and Rao, 1978, 1983, 1984; Papathanassiou and King, 1983; Rao and Doughtie, 1983; Papathanassiou, 1985; Freire and McNamara, 1995; McNamara and Lima, 1997; McNamara and Torres, 1999).

The general structure of the gill lamellae in *R. exoculata* is similar to the corresponding organization in *P. adspersus*. However, in *R. exoculata*, the axial zone of the epithelial cells contains fewer mitochondria and does not reveal the presence of Na^+,K^+-ATPase. Thus the axial epithelium of the gills of *R. exoculata* does not seem involved in active ion exchanges.

The lateral expansions of the epithelial cells of the gills of *P. adspersus* and *R. exoculata* are similar, thin, poorly differentiated and covered by a thin cuticle. They lack Na^+,K^+-ATPase in both species. They present the features of respiratory epithelia as described in other species, e.g. (i) in the anterior and posterior gills of osmoconforming crabs as *Cancer pagurus* (Péqueux et al., 1988), (ii) in the anterior gills of osmoregulating crabs as *Callinectes sapidus* (Copeland and Fitzjarrell, 1968), *Eriocheir sinensis* (Barra et al., 1983; Péqueux et al., 1988), *Carcinus maenas* (Compère et al., 1989; Goodman and Cavey, 1990), and *Gecarcinus lateralis* (Copeland, 1968), (iii) in the gills of the lobster *Homarus gammarus* (Haond et al., 1998) and in some filaments of the gills of the crayfish *Astacus leptodactylus*, *Austropotamobius pallipes* (Dunel-Erb et al., 1982, 1997) and *Procambarus clarkii* (Dickson et al., 1991).

In summary, the gills of *P. adspersus* seem to have a double function, respiration and osmoregulation, respectively effected by the lateral expansions and the axial part of the cells. This situation is different from, but reminiscent of the functional separation between the respiratory anterior gills and the osmoregulatory posterior gills in brachyuran crabs (Copeland and Fitzjarrell, 1968; Barra et al., 1983; Péqueux et al., 1988; Compère et al., 1989) and between different filaments of the same gill in crayfish (Barradas et al., 1999a).

The gills of *R. exoculata* appear involved in respiration only. Compared to the situation in epibenthic species, their single function may be interpreted as an adaptation to the relative hypoxia of the deep hydrothermal environment. This hypothesis is supported by the higher number of gills in *R. exoculata* compared to *P. adspersus* (10 pairs versus 8 pairs) and by the comparatively high oxygen affinity of the hemocyanin in *R. exoculata* (Lallier and Truchot, 1997; Lallier et al., 1998). The gills of *R. exoculata* present other peculiarities such as the presence of bacteria on the cuticle, the thickness of their epithelium, the presence of black particules in perinuclear areas, the mitochondrial granules and the reduced and disorganized mitochondrial crests. They might represent other adaptations to their particular environment such as sulfide detoxication for some of them. This detoxication function has already been hypothesized in the gills of *R. exoculata* through sulfide-oxidising bodies (SOBs) (Compère et al., 2002). Mitochondria containing small dense granules have also been reported in the gills of the "Pompeii worms" *Alvinella pompejana* and *Paralvinella grasslei* (Jouin and Gaill, 1990) and in the meiobenthic thiobiotic turbellarian *Solenofilomorpha funilis*, a species able to detoxify sulfides (Duffy and Tyler, 1984).

4.2. Epipodites

In *P. adspersus* and *R. exoculata*, the epipodites present two thick lateral epithelial layers without pillar structures, separated by an axial septum presenting small hemolymph lacunae. This structure is similar to those described in *Crangon crangon* (Debaisieux, 1970) and in the peneid shrimp *Peneus japonicus* (Bouaricha et al., 1994). The epipodites of the lobster *Homarus gammarus* (Haond et al., 1998) and the lamina (equivalent structure) of the crayfish *Astacus leptodactylus* and *Austropotamobius pallipes* (Dunel-Erb et al., 1982, 1997) present the same organization of two facing epithelia, but with the addition of frequent pillar cells and of a voluminous central lacuna.

The epithelial cells of the epipodites of *P. adspersus* and *R. exoculata* are typical ionocytes displaying apical microvilli and numerous mitochondria closely associated with deep basal infoldings which reveal a strong presence of Na^+,K^+-ATPase. Ionocytes have also been described in the epipodites of *Peneus japonicus* (Bouaricha et al., 1994), *Austropotamobius pallipes* and *Astacus leptodactylus* (Dunel-Erb et al., 1982, 1997) and *Homarus gammarus* (Haond et al., 1998). The presence of Na^+,K^+-ATPase was also detected in the last species (Lignot et al., 1999). The epipodites of *P. adspersus* and *R. exoculata* may both have an osmoregulatory function. Compared to *P. adspersus*, the epipodites of *R. exoculata* seem to present particular features potentially associated with their function in the deep-sea hydrothermal

vents. Well-developed apical microvilli are present in both species: in *R. exoculata* they are associated with numerous vesicules, which are scarce or even absent in *P. adspersus* (this study) and in *Palaemon serratus* (Papathanassiou and King, 1983). These vesicules have been interpreted as pinocytic vesicules (Copeland and Fitzjarrell, 1968; Bubel and Jones, 1974; Bubel, 1976; Papathanassiou and King, 1983). Their membranes in *R. exoculata* are close to numerous mitochondria and thus may constitute "mitochondrial pumps" as it has been hypothesized by Maina (1990) in the crab *Potamon niloticus*. The epithelia of the epipodites of *R. exoculata* and *P. adspersus* may thus play a role in osmoregulation and also in other ionic exchanges, e.g. in acid-base regulation (Truchot, 1983; Henry and Wheatly, 1992).

4.3. Branchiostegites

The branchiostegites of *P. adspersus* and *R. exoculata* present cellular structures similar to those described in the same organs in the thalassinid *Callianassa jamaicense* (Felder et al., 1986), *Peneus aztecus* (Talbot et al., 1972), *P. japonicus* (Bouaricha et al., 1994) and *Homarus gammarus* (Haond et al., 1998): two thick and irregular lateral layers of epithelium separated by pillar cells which cross a voluminous central hemolymph lacuna. To our knowledge, our study is the only one concerning this site in caridean shrimps.

The internal epithelium of the branchiostegites of *P. adspersus* reveals the ultrastuctural features of typical ionocytes associated to a strong immunolabelling for Na^+,K^+-ATPase. The presence of this enzyme has been already reported in the branchiostegites of juvenile *Homarus gammarus* (Lignot et al., 1999; Lignot and Charmantier, 2001). It points to an involvement of these structures in osmoregulation in the lobster and the shrimp.

In *R. exoculata*, the inner epithelium of the branchiostegite, covered by a thin cuticle, comprises high prismatic cells presenting apical microvilli and numerous mitochondria but with only short and scarce basal infoldings and no evidence of Na^+,K^+-ATPase. The branchiostegites of *R. exoculata* are therefore probably not involved in active sodium transport.

The branchiostegites of *P. adspersus* and *R. exoculata* have therefore different functions. They most probably are osmoregulatory organs in *P. adspersus*. Their role in *R. exoculata*, still hypothetical, may be linked to the presence of the large basal protuberances filled with black particles. They could participate to sulfide detoxication in addition to the gills.

4.4. Localization of the osmoregulatory structures

Since the early studies on crustacean osmoregulation, the gills have been considered as the primary site for ionic and osmotic regulation (review in Robertson, 1960; Lockwood, 1962; Gilles, 1975; Croghan, 1976; Mantel and Farmer, 1983; Péqueux et al., 1984; Towle, 1984; Péqueux, 1995). In some species, osmoregulation is anatomically separated from the respiratory function. In strongly osmoregulating brachyurans such as *Pachygrapsus marmoratus*, the anterior gills are specialized in respiration and the posterior gills have an osmoregulatory function (Péqueux, 1995). In these species, the epipodites and branchiostegites do not display any osmoregulatory structure. In crayfish, on each gill, the filaments are specialized either in ionic regulation or in gas exchanges (Taylor and Taylor, 1992; Barradas et al., 1999a,b). A few studies have revealed that differentiated osmoregulatory tissues can be present in the branchial cavity of some decapods, located at two sites different from the gills. These sites include the branchiostegites (Talbot et al., 1972; Felder et al., 1986; Bouaricha et al., 1994; Haond et al., 1998; Lignot et al., 1999) and/or the epipodites (Dunel-Erb et al., 1982, 1997; Kikuchi and Matsumasa, 1993; Bouaricha et al., 1994; Haond et al., 1998; Barradas et al., 1999b; Lignot et al., 1999). They may be temporary at certain stages of development. The observations reported here regarding *P. adspersus* and *R. exoculata* bring additional structural and functional evidence of the existence of extrabranchial ion-transporting tissues in adult decapod crustaceans. *P. adspersus* is a strong hyper-hypo-osmoregulatory species (Martinez, 2001) like most epibenthic caridaea (Mantel and Farmer, 1983). Its capacity to osmoregulate originates from a combined activity of the gills, the epipodites and the branchiostegites. The two latter sites might play a predominant role if one considers their high number of typical ionocytes including a high content of Na^+,K^+-ATPase. They probably are the major site for active ionic exchanges in this shrimp. In the gills, the axial zone of the epithelial cells, similar to atypical ionocytes and with Na^+,K^+-ATPase, may also participate to active ionic exchanges, whereas the lateral expansions would be involved in respiration. In *R. exoculata*, ionocytes and Na^+,K^+-ATPase have been found only in the epipodites, and not along the gills and the branchiostegites. We hypothesize that the ability to osmoregulate in *R. exoculata* is comparatively low, considering the small surface of the six epipodites compared to those of the gills and/or the branchiostegites in strong osmoregulating decapods including epibenthic shrimps.

This species may have favoured the repiratory mechanism at the expense of the osmotic regulation in order to deal with the hypoxia of the deep hydrothermal environment. Their frequent movements probably maintain the shrimps in an environment where temperature conditions (recently determined to be below 25 °C by Ravaux et al., 2003) and salinity are both within tolerable range. These constant movements are also favorable for the search of food. This set of hypothesis seems in agreement with in situ observations which describe the shrimps *R. exoculata* as very and constantly active animals, all the more active as they get closer to the warmest emission sources (Segonzac et al., 1993; Van Dover et al., 1988, 1996; Sarradin et al., 1999). The structural evidence for limited osmoregulation in *R. exoculata* reported here should be complemented by direct measurements of hemolymph osmolality

on freshly captured shrimps, exposed on board to different salinities in pressurized aquaria (Ravaux et al., 2003). It will be a nice step to understand the extent of the osmoregulatory adaptations of the caridean vent shrimps.

Acknowledgements

The authors wish to thank Dr. D.M. Fambrough for his generous gift of the anti-Na^+,K^+-ATPase antibody, Drs. M. Segonzac, B. Shillito and J.-Y. Toullec for the fixations on board, the chief scientists of the different cruises, and E. Grousset, C. Blasco, J.-P. Selzner, P. Azais and F. Aujoulat for their technical assistance.

References

Allen, E.J., 1892. On the minute structure of the gills of *Palaemonetes varians*. Q.J. Microsc. Sci. 34, 75–84.

Barra, J.A., Péqueux, A., Humbert, W., 1983. A morphological study on gills of a crab acclimated to fresh water. Tissue Cell 15, 583–596.

Barradas, C., Dunel-Erb, S., Lignon, J., Péqueux, A., 1999a. Superimposed morphofunctional study of ion regulation and respiration in single gill filaments of the crayfish *Astacus leptodactylus*. J. Crust. Biol. 19, 14–25.

Barradas, C., Wilson, J.M., Dunel-Erb, S., 1999b. Na^+/K^+-ATPase activity and immunocytochemical labelling in podobranchial filament and lamina of the freshwater crayfish *Astacus leptodactylus* Escscholtz: evidence for the existence of sodium transport in the filaments. Tissue Cell 31, 523–528.

Bouaricha, N., Charmantier-Daures, M., Thuet, P., Trilles, J.-P., Charmantier, G., 1994. Ontogeny of osmoregulatory structures in the shrimp *Penaeus japonicus* (Crustacea Decapoda). Biol. Bull. 186, 29–40.

Bubel, A., 1976. Histological and electron microscopical observations on the effects of different salinities and heavy metal ion, on the gills of *Jaera nordmanni* (Rathke) (Crustacea Isopoda). Cell Tissue Res. 167, 65–95.

Bubel, A., Jones, M.B., 1974. Fine structure of the gills of *Jaera nordmanni* (Rathke) (Crustacea Isopoda). J. Mar. Biol. Ass. UK 54, 737–743.

Charmantier, G., 1998. Ontogeny of osmoregulation in crustaceans: a review. Invert. Reprod. Dev. 33, 177–190.

Chevaldonné, P., 1997. The fauna of deep-sea hydrothermal vents: an introduction. In: Desbruyères, D., Segonzac, M. (Eds.), Handbook of Deep-Sea Hydrothermal Vent Fauna. InterRidge/IFREMER, Brest, pp. 7–20.

Christoffersen, M.L., 1986. Phylogenetic relationships between Oplophoridae, Atyidae, Pasiphaeidae, Alvinocarididae fam. n., Bresiliidae, Psalidopodidae and Disciadidae (Crustacea Caridea Atyoidea). Bol. Zool. Univ. S. Paulo 10, 273–281.

Compère, P., Wanson, S., Péqueux, A., Gilles, R., Goffinet, G., 1989. Ultrastructural changes in the gill epithelium of the green crab *Carcinus maenas* in relation to the external salinity. Tissue Cell 21, 299–318.

Compère, P., Martinez, A.-S., Charmantier-Daures, M., Toullec, J.-Y., Goffinet, G., Gaill, F., 2002. Does sulfide detoxication occur in the gills of the hydrothermal vent shrimp *Rimicaris exoculata*? C.R. Acad. Sci. 325, 1–6.

Copeland, D., 1968. Fine structure of salt and water uptake in the land crab, *Gecarcinus lateralis*. Am. Zool. 8, 417–432.

Copeland, D., Fitzjarrell, A.T., 1968. The salt absorbing cells in the gills of the blue crab (*Callinectes sapidus* Rathbun) with notes on modified mitochondria. Z. Zellforsch. 92, 1–22.

Croghan, P.C., 1976. Ionic and osmotic regulation of aquatic animals. In: Blight, J., Cloudsley-Thomson, J.L., McDonald, A.G. (Eds.), Environmental Physiology of Animals. Oxford University Press, New York, Oxford, pp. 59–94.

Cuénot, L., 1893. Etudes physiologiques sur les crustacés décapodes. Arch. Biol. 13, 245–303.

Debaisieux, P., 1970. Appareil branchial de *Crangon vulgaris* (Décapode nageur). Anatomie et histologie. La Cellule XIX, 64–77.

Dickson, J.S., Dillaman, R.M., Roer, R.D., Roye, D.B., 1991. Distribution and characterization of ion transporting and respiratory filaments in the gills of *Procambarus clarkii*. Biol. Bull. 180, 154–166.

Doughtie, D.G., Rao, K.R., 1978. Ultrastructural changes induced by sodium pentachlorophenate in the grass shrimp *Palaemonetes pugio*, in relation to the molt cycle. In: Rao, K.R. (Ed.), Pentachlorophenol: Chemistry, Pharmacology and Environmental Toxicology. Plenum Press, New York, pp. 213–250.

Doughtie, D.G., Rao, K.R., 1983. Ultrastructural and histological study of degenerative changes leading to black gills in grass shrimp exposed to a dithiocarbamate biocide. J. Invert. Pathol. 41, 33–50.

Doughtie, D.G., Rao, K.R., 1984. Histopathological and ultrastructural changes in the antennal gland, midgut, hepathopancreas, and gill of the grass shrimp following exposure to hexavalent chromium. J. Invert. Pathol. 43, 89–108.

Drach, P., Tchernigovtzeff, C., 1967. Sur la méthode de détermination des stades d'intermue et son application générale aux crustacés. Vie et Milieu 18A, 595–610.

Duffy, J.E., Tyler, S., 1984. Quantitative differences in mitochondrial ultrastructure of a thiobiotic and oxybiotic turbellarian. Mar. Biol. 83, 95–102.

Dunel-Erb, S., Massabuau, J.C., Laurent, P., 1982. Organisation fonctionnelle de la branchie d'écrevisse. C.R. Soc. Biol. 176, 248–258.

Dunel-Erb, S., Barradas, C., Lignon, J., 1997. Morphological evidence for the existence of two distinct types of mitochondria rich cells in the gills of the crayfish *Astacus leptodactylus* Eschscholtz. Acta Zool. 78, 195–203.

Farelly, C., Greenaway, P., 1992. Morphology and ultrastructure of the gills of terrestrial crabs (Crustacea Gecarcinidae and Grapsidae): adaptations for air-breathing. Zoomorphol. 112, 39–50.

Felder, J.M., Felder, D.L., Hand, S.C., 1986. Ontogeny of osmoregulation in the estuarine ghost shrimp *Callianassa jamaicense* var. *louisianensis* Schmitt (Decapoda Thalassinidea). J. Exp. Mar. Biol. Ecol. 99, 91–105.

Finol, H.J., Croghan, P.C., 1983. Ultrastructure of the branchial epithelium of an amphibious brackish-water crab. Tissue Cell 15, 63–75.

Freire, C.A., McNamara, J.C., 1995. Fine structure of the gills of the fresh-water shrimp *Macrobrachium olfersii* (Decapoda): effect of acclimation to high salinity medium and evidence for involvement of the lamellar septum in ion uptake. J. Crust. Biol. 15, 103–116.

Galkin, S.V., Moskalev, L.I., 1990. Hydrothermal fauna of the Mid-Atlantic Ridge. Oceanol. 30, 624–627.

Gebruk, A.V., Galkin, S.V., Vereshchaka, A.L., Moskalev, L., Southward, A.J., 1997. Ecology and biogeography of the hydrothermal vent fauna of the Mid-Atlantic Ridge. Adv. Mar. Biol. 32, 93–143.

Gilles, R., 1975. Mechanism of ion transport and osmoregulation. In: Kinne, O. (Ed.), Marine Ecology, vol. 2. Wiley-Interscience, New York, pp. 259–347.

Goodman, S.H., Cavey, M.J., 1990. Organization of a phyllobranchiate gill from the green shore crab *Carcinus maenas* (Crustacea Decapoda). Cell Tissue Res. 260, 495–505.

Haond, C., Flik, G., Charmantier, G., 1998. Confocal laser scanning and electron microscopical studies on osmoregulatory epithelia in the branchial cavity of the lobster *Homarus gammarus*. J. Exp. Biol. 201, 1817–1833.

Henry, R.P., Wheatly, M.G., 1992. Interaction of respiration, ion regulation, and acid-base balance in the everyday life of aquatic crustaceans. Am. Zool. 32, 407–416.

Jouin, C., Gaill, F., 1990. Gills of hydrothermal vent annelids: structure, ultrastructure and functional implications in two alvinellid species. Prog. Oceanogr. 24, 59–69.

Kikuchi, S., Matsumasa, M., 1993. Two ultrastructurally distinct types of transporting tissues, the branchiostegal and the gill epithelia, in an estuarine tanaid, *Sinelobus stanfordi* (Crustacea Peracarida). Zoomorphol. 113, 253–260.

Lakin, R.C., Jinks, R.N., Battelle, B.-A., Herzog, E.D., Kass, L., Renninger, G.H., Chamberlain, S.C., 1997. Retinal anatomy of *Chorocaris chacei*, a deep-sea hydrothermal vent shrimp from the mid-Atlantic ridge. J. Comp. Neurol. 385, 503–514.

Lallier, F.H., Truchot, J.-P., 1997. Hemocyanin oxygen-binding properties of a deep-sea hydrothermal vent shrimp: evidence for a novel cofactor. J. Exp. Zool. 277, 357–364.

Lallier, F.H., Camus, L., Chausson, F., Truchot, J.-P., 1998. Structure and function of hydrothermal vent crustaceans haemocyan: an update. Cah. Biol. Mar. 39, 313–316.

Lignot, J.-H., Charmantier-Daures, M., Charmantier, G., 1999. Immunolocalization of Na^+,K^+-ATPase in the organs of the branchial cavity of the European lobster *Homarus gammarus* (Crustacea Decapoda). Cell Tissue Res. 296, 417–426.

Lignot, J.-H., Charmantier, G., 2001. Immunolocalization of Na^+,K^+-ATPase in the branchial cavity during the early development of the European lobster *Homarus gammarus* (Crustacea Decapoda). J. Histochem. Cytochem. 49, 1013–1023.

Lockwood, A.P.M., 1962. The osmoregulation of crustacea. J. Exp. Biol. 37, 257–305.

Lucu, C., Flik, G., 1999. Na^+-K^+-ATPase, Na^+/Ca^{2+} exchange activities in gills of hyperregulating *Carcinus maenas*. Am. J. Physiol. 276, 490–499.

Maina, J.N., 1990. The morphology of the gills of the freshwater African crab *Potamon niloticus* (Crustacea, Brachyura, Potamonidae). A scanning and transmission electron microscopic study. J. Zool. Lond. 221, 199–515.

Mantel, L.H., Farmer, L.L., 1983. Osmotic and ionic regulation. In: Mantel, L.H. (Ed.), The Biology of Crustacea: Internal Anatomy and Physiological Regulation, vol. 5. Academic Press, New York, pp. 53–161.

Martinez, A.-S., 2001. Adaptations morpho-fonctionnelles des crustacés caridés et brachyoures à la salinité du milieu hydrothermal profond. Thèse de Doctorat, Université Montpellier II, pp. 178.

Martoja, R., Martoja-Pierson, M., 1967. Initiation aux techniques de l'histologie animale. Masson et Cie, Paris.

McNamara, J.C., Lima, A.G., 1997. The route of ion and water movements across the gill epithelium of the freshwater shrimp *Macrobrachium olfersii* (Decapoda Palaemonidae): evidence from ultrastructural changes induced by acclimation to saline media. Biol. Bull. 192, 321–331.

McNamara, J.C., Torres, A.H., 1999. Ultracytochemical location of Na^+/K^+-ATPase activity and effect of high salinity acclimation in gill and renal epithelia of the freshwater shrimp *Macrobrachium olfersii* (Crustacea Decapoda). J. Exp. Zool. 284, 617–628.

Papathanassiou, E., King, P.E., 1983. Ultrastructural studies on the gills of *Palaemon serratus* (Pennant) in relation to cadmium accumulation. Aquat. Toxicol. 3, 273–284.

Papathanassiou, E., 1985. Effects of cadmium ions on the ultrastructure of the gill cells of the brown shrimp *Crangon crangon* (L.) (Decapoda Caridea). Crustaceana 48, 6–17.

Patil, H.S., Kaliwal, M.B., 1989. Histopathological effects of zinc on the gills of prawn *Macrobrachium hendersodyanum*. Z. Angew. Zool. 76, 505–509.

Péqueux, A., Marchal, A., Wanson, S., Gilles, R., 1984. Kinetic characteristics and specific activity of gill (Na^+/K^+)ATPase in the euryhaline chines crab, *Eriocheir sinensis* during salinity acclimation. Mar. Biol. Lett. 5, 35–45.

Péqueux, A., Gilles, R., Marshall, W.S., 1988. NaCl transport in gills and related structures. Part I: Invertebrates. In: Greger, R. (Ed.), Advances in Comparative and Environmental Physiology: NaCl Transport in Epithelia, vol. 1. Springer Verlag, Berlin, Heidelberg, New York, pp. 1–73.

Péqueux, A., 1995. Osmotic regulation in crustaceans. J. Crust. Biol. 15, 1–60.

Pottu-Boumendil, J., 1989. Techniques en microscopie électronique. In: Principes et méthodes de préparation. INSERM, Paris.

Ravaux, J., Gaill, F., Le Bris, N., Sarradin, P.-M., Jollivet, D., Shillito, B., 2003. Heat-shock response and temperature resistance in the deep-sea shrimp *Rimicaris exoculata*. J. Exp. Biol. 206, 2345–2354.

Rao, K.R., Doughtie, D.G., 1983. Histopathological changes in grass shrimp exposed to chromium, pentachlorophenol and dithiocarbamates. Resp. Mar. Organ. Pollut. International symposium on responses of marine organism to pollutants, Woods Hole, USA, 1983, pp. 371–395.

Robertson, J.D., 1960. Osmotic and ionic regulation. In: Waterman, T.H. (Ed.), Physiology of Crustacea, vol. 1. Academic Press, New York, pp. 317–339.

Sarradin, P.-M., Caprais, J.-C., Briand, P., Gaill, F., Shillito, B., Desbruyères, D., 1998a. Chemical and thermal description of environment of the Genesis hydrothermal vent community (13°N EPR). Cah. Biol. Mar. 39, 159–167.

Sarradin, P.-M., Caprais, J.-C., Riso, R., Comtet, T., Aminot, A., 1998b. Brief account of the chemical environment at hydrothermal vent mussel beds on the MAR. Cah. Biol. Mar. 39, 253–254.

Sarradin, P.-M., Caprais, J.-C., Riso, R., Kerouel, R., Aminot, A., 1999. Chemical environment of the hydrothermal mussel communities in the Lucky Strike and Menez Gwen vent fields, Mid Atlantic Ridge. Cah. Biol. Mar. 40, 93–104.

Segonzac, M., De Saint Laurent, M., Casanova, B., 1993. L'énigme du comportement trophique des crevettes Alvinocarididae des sites hydrothermaux de la dorsale médio-Atlantique. Cah. Biol. Mar. 34, 535–571.

Takeyasu, K., Tamkun, M.M., Renaud, K.J., Fambrough, D.M., 1988. Ouabain sensitive (Na^+, K^+)-ATPase activity expressed in mouse L cells by transfection with DNA encoding the α-subunit of an avian sodium pump. J. Biol. Chem. 263, 4347–4354.

Talbot, P., Clark, W.H., Lawrence, A.L., 1972. Light and electron microscopic studies on osmoregulatory tissue in the developing brown shrimp, *Penaeus aztecus*. Tissue Cell 4, 271–286.

Taylor, H.H., Taylor, E.W., 1992. Gills and lungs: the exchange of gases and ions. In: Harrisson, F.W., Humes, A.G. (Eds.), Microscopic Anatomy of Invertebrates: Decapoda Crustacea, vol. 10. John Wiley and Sons, New York, pp. 203–293.

Towle, D.W., 1984. Regulatory functions of Na^+-K^+ATPase in marine and estuarine animals. In: Péqueux, A., Gilles, R., Bolis, L. (Eds.), Osmoregulation in Estuarine and Marine Animals. Springer Verlag, Berlin, Heidelberg, New York, pp. 157–170.

Truchot, J.-P., 1983. Regulation of acid-base balance. In: Bliss, D.E. (Ed.), The Biology of Crustacea: Internal Anatomy and Physiological Regulations, vol. 5. Academic Press, London, pp. 431–457.

Truchot, J.-P., Lallier, F.H., 1998. High CO_2 content in hydrothermal vent water at the Snake Pit area, Mid-Atlantic Ridge. Cah. Biol. Mar. 39, 153–158.

Tunnicliffe, V., McArthur, A.G., McHugh, D., 1998. A biogeographical perspective of the deep-sea hydrothermal vent fauna. Adv. Mar. Biol. 34, 353–442.

Van Dover, C.L., Fry, B., Grassle, J.F., Humphris, S., Rona, P.A., 1988. Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-Atlantic ridge. Mar. Biol. 98, 209–216.

Van Dover, C.L., 1995. Ecology of the Mid-Atlantic ridge hydrothermal vents. In: Parson, L.M., Walker, C.L., Dixon, D.R. (Eds.), Hydrothermal Vents and Processes, vol. 87. Geological Society, Special Publication, pp. 257–294.

Van Dover, C.L., Desbruyères, D., Segonzac, M., Comtet, T., Saldanha, L., Fiala-Medioni, A., Langmuir, C., 1996. Biology of the Lucky Strike hydrothermal field. Deep-Sea Res. 43, 1509–1529.

Van Dover, C.L., 2000. The Ecology of Deep-Sea Hydrothermal Vents. Princeton University Press, Princeton.

Ziegler, A., 1997. Immunocytochemical localization of Na^+, K^+ ATPase in the calcium transporting sternal epithelium of the terrestrial Isopod *Porcellio scaber* L. (Crustacea). J. Histochem. Cytochem. 45, 437–446.