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Architecture of, and water circulation and flow rate in, the house of the planktonic tunicate *Oikopleura labradoriensis*

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Abstract. The gelatinous house of *Oikopleura labradoriensis* (Tunicata, Appendicularia), collected from the docks of Friday Harbor Laboratories, University of Washington, USA, in 1984, 1986 and 1990, was examined in vivo by stereomicroscopy and strobe-light macrophotography, and after fixation and processing for light and electron microscopy. In addition to confirming previous knowledge and adding new information on structural organization of the oikopleurid house, this study presents quantitative data on important aspects of its function. Particles small enough to pass through the inlet filters (pore width ~13 µm) were concentrated between differently constructed upper and lower food-concentrating filters (pore widths 0.18 and 0.24 µm, respectively). These filtes were held together by an intermediary screen of widely separated ribbon-like filaments. Water sieved through the filters left the house through a pressure-regulated exit valve. However, the intermittent activity of the tail pump and the elasticity of the house caused frequent refluxes of water that cleared both inlet filters and food-concentrating filter screens of adhering particles. During these refluxes the food-concentrating filters usually collapsed and compacted the trapped particles into coarser aggregates. With each pumping cycle the particles and aggregates were brought closer to the midline. From here they were periodically drained into the mouth of the organism through a medial food-collecting tube, to be recaptured in a pharyngeal feeding filter secreted by the organism's endostyle. Based on the size and movements of the tail within the close-fitting tail chamber, a water flow rate of ~0.84 ml min^{-1} was calculated for medium-sized houses (belonging to individuals with trunk length of ca. 1.2 mm). Taking the intermittent pumping activity of the tail into account, this equals ~35 ml h^{-1}. Flow through the food-collecting tube was ~1 µl min^{-1}, laminar and intermittent, and was probably comparable to a rate of ~0.04 ml h^{-1}. Accordingly, the house allowed the oikopleurid to feed on a ca. 1000 × concentrated suspension of particles. Water speed through the meshes of the food-concentrating filters was ca. 0.15 mm min^{-1}, or 2.5 µm s^{-1}.

Introduction

Appendicularians of the Oikopleuridae family (Lohmann, 1915) are common and abundant marine planktonic tunicates. They consist of a roundish trunk and an elongated muscular tail, and usually surround themselves with a completely transparent, jelly-like house (Mertens 1830, Fol 1872). This house is secreted by the epidermal cells covering the anterior part of the trunk (Lohmann 1896), the so-called oikoblast (house-forming) epithelium (Klaatsch 1895). The secretory product of these cells, the house rudiment, stays closely attached to the trunk until the organism needs it. The rudiment is then inflated with water and expanded by undulatory movements of the tail until the whole organism is lodged within a complex gelatinous sphere (Fenaux and Hirel 1972).

This house is an elaborate particle trap through which the animal pumps water, and from which the animal sucks particle-enriched water into its pharynx. Numerous studies exist on the morphology and function of these houses (e.g. Lohmann 1899, 1933/56, Körner 1952, Jørgensen 1966, Alldredge 1976a, 1977, Flood 1978, 1981, 1983, 1991a, Deibel et al. 1985, Deibel 1986, Fenaux 1986, Deibel und Powell 1987a, Flood et al. 1990). However, many details have yet to be disclosed. Firstly, the way in which water is led through the different chambers and passages of the house remains a matter of controversy. Secondly, the exact way in which water is filtered through the food-concentrating filter system remains obscure. Thirdly, the amount of water filtered through the house, as well as its concentration factor for food particles, is still unknown. In addition, the structure of the *Oikopleura labradoriensis* house has never been described before. The present study aims at increasing our understanding of these aspects of the oikopleurid house.

Materials and methods

The appendicularian *Oikopleura labradoriensis* (Lohmann 1896) was collected from the docks at Friday Harbor Laboratories, Uni-

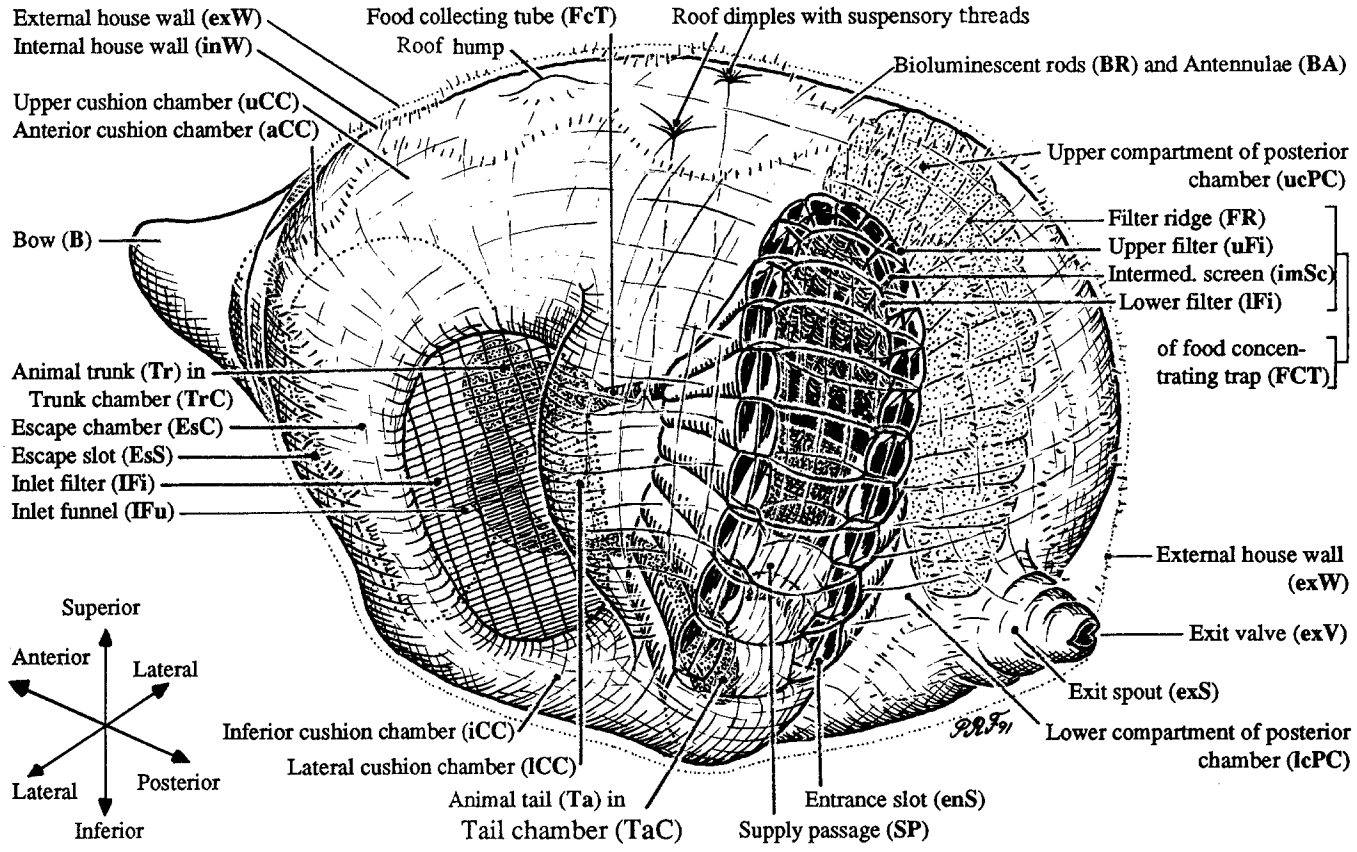

Fig. 1. *Oikopleura labradoriensis*. Diagram of organism in its house, with axes of orientation

versity of Washington, USA, in January and February 1984, 1986 and 1990. Specimens in intact houses were visually identified at night using a beam of light scanning the superficial layer of water horizontally. They were then scooped into glass vials. Specimens in their houses were also obtained by keeping undamaged organisms (without houses) overnight at room temperature in gently agitated glass jars with fresh seawater filtered through a 0.2-µm-pore filter.

Living specimens in their houses were examined and photographed through a Wild M400 photomacroscope in either bright or dark field illumination. Two electronic flashes, mounted either below or above the specimen stage, served as light sources so as to obtain sufficiently short exposure times. Houses examined in bright field mode were prestained with carmine particles (Lohmann 1899) and/or the natural ink of the cuttlefish *Sepia officinalis* suspended in seawater (Flood et al. 1990).

An electronic strobe flashlight from Chadwick-Helmuth Co., Inc., operating at 1000 or 600 flashes min^{-1} and exposure times of ¼ to 2 s, was used to make multiple-exposed photographs through the same macroscope. The distance between points in individual particle tracks was measured and used to calculate current speeds; the same macrographs were used to measure the size or cross-sectional area of relevant passages in order to derive flow rate values. Attempts were also made to monitor the jet of water leaving some houses, using a heated thermocouple located less than 2 mm behind the exit spout. Although the probe (~1.5 mm diameter) was far too large to reveal details of flow rate, the cooling effect of the jet revealed the organism's pumping pattern. All vital studies and timings were made for specimens in seawater of 11 to 14 °C.

Some jettisoned houses were mounted (wet) for fluorescence or differential interference contrast light microscopy. Others were dried onto slides (Flood 1991a), stained, and mounted for bright field light microscopy. Ruthenium Red (1% in 50-m*M* cacodylate-buffered 60% seawater), Toluidine Blue (1% in 0.1 *M* sodium borate) and Alcian Blue (1% in 0.6 *M* sucrose) were used as stains. Some were fixed in 2% formaldehyde and 1% glutaraldehyde in 50-m*M* cacodylate buffer and 60% seawater, followed by 1% osmium tetroxide in the same buffered saline. These were processed by microdissection and critical-point or air drying for direct transmission electron microscopy (TEM); by dehydration, plastic embedding and ultramicrotomy for thin-section TEM; and by dehydration, critical-point drying and gold/palladium sputter-coating for scanning electron microscopy (SEM). For direct TEM, Pt/C was evaporated from a 20° angle to create a shadow, or samples were stained by Ruthenium Red as described above. Stereoscopic picture pairs were obtained by tilting the specimen stage of the microscopes.

Results

In describing the house of *Oikopleura labradoriensis* it is necessary to relate common anatomical terms (such as lateral, anterior, superior, etc.) to the usual orientation of the house in the sea (Alldredge 1976b), rather than to the direction of the organism as suspended within the house (Fig. 1). Terms such as dorsal, ventral, cranial and caudal should be avoided, as their interpretation is ambiguous in the case of larvaceans, which have reflexed and twisted tails. The nomenclature used here (Fig. 1) is the same as proposed by Flood (1983) for *O. dioica* houses. This is mostly a direct translation of the german terms used by Lohmann (1933/1956).

Size and external shape of house

The house of *Oikopleura labradoriensis* was elongated, its length being about 1.5 times its width and height (Figs. 2

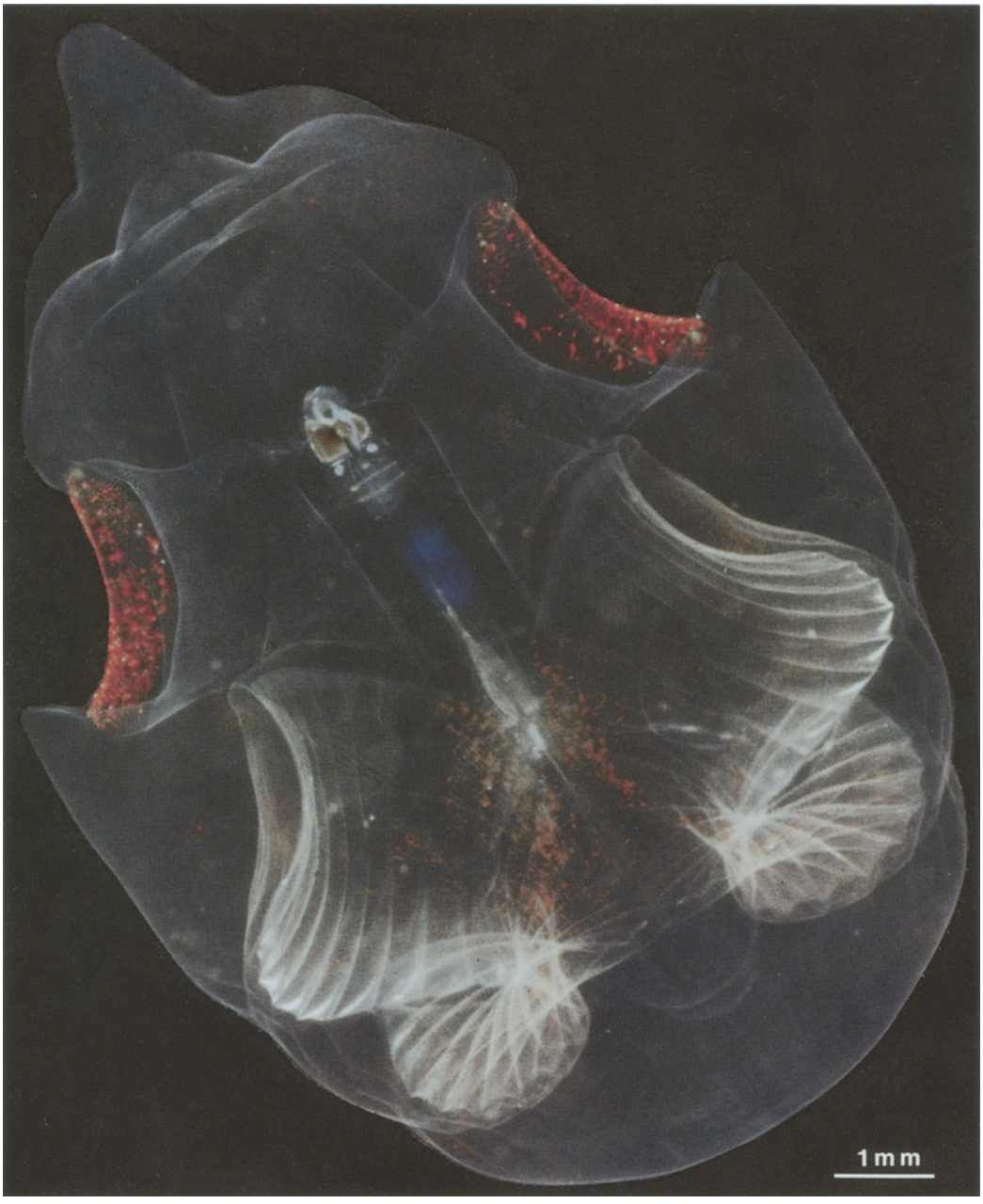

Fig. 2. *Oikopleura labradoriensis*. The tunicate is shown here in its house after staining with carmine particles and *Sepia officinalis* ink. See Figs. 1 and 3A for identification of structural components

and 3), and about 2 times as long as the organism itself. The house length ranged between 5 and 18 mm, depending on size and age of the organism. In this study mainly houses of 10 to 14 mm length were examined.

At present we have no good way of visualizing the real external walls of inhabitated oikopleurid houses. Their location was inferred from scattered adhering particles (cf. Flood 1991 a). Except for a prominent bow, where the external wall was separated from the periphery of the internal chambers by ~ 2 mm, the distance between the real external surface of the house and the peripheral surface of the internal chambers was quite limited (< 1 mm).

The external walls consisted of a 3- to 10-nm-thick web of filaments. Although ordinary TEM revealed highly variable diameter and apparent branching and joining points between these filaments (Fig. 4a, b), the samples shadowed by Pt/C and the SEM specimens revealed more evenly thick, straighter, randomly oriented filaments, without prominent branching and joining points (Fig. 4c, d). In all samples the filaments ranged in thick-

Fig. 3. *Oikopleura labradoriensis*. Dark field macrographs of an inhabitated house stained by filtration of *Sepia officinalis* ink and carmine in (A) top, (B) frontal, and (C) lateral projection. Note the inreased size of food particles near the midline of the food-concentrating trap in (A) and (B), and the attachment of the trunk in the wall separating the escape chamber from the trunk chamber (arrows). Abbreviations as in Fig. 1

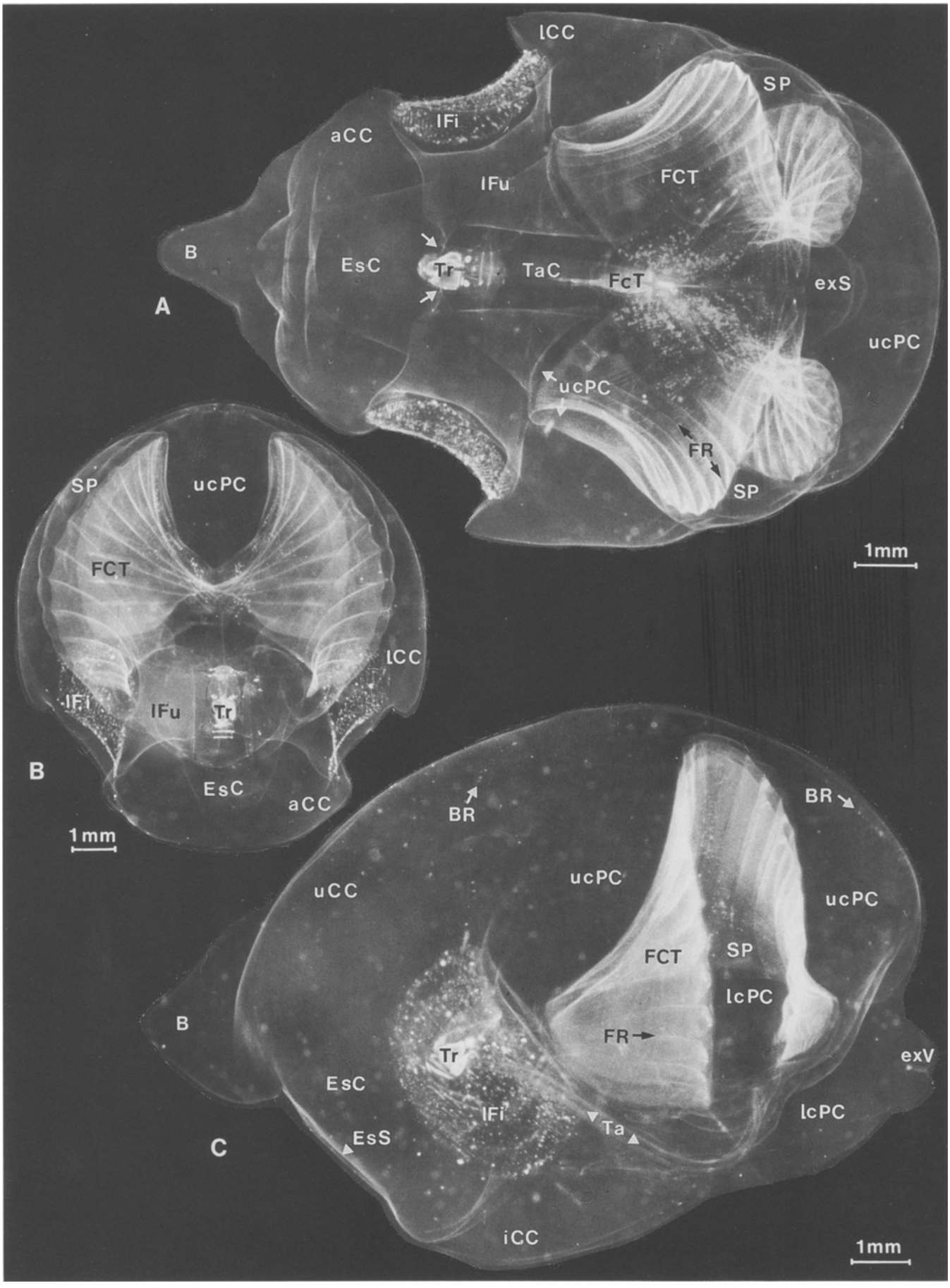
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Fig. 3

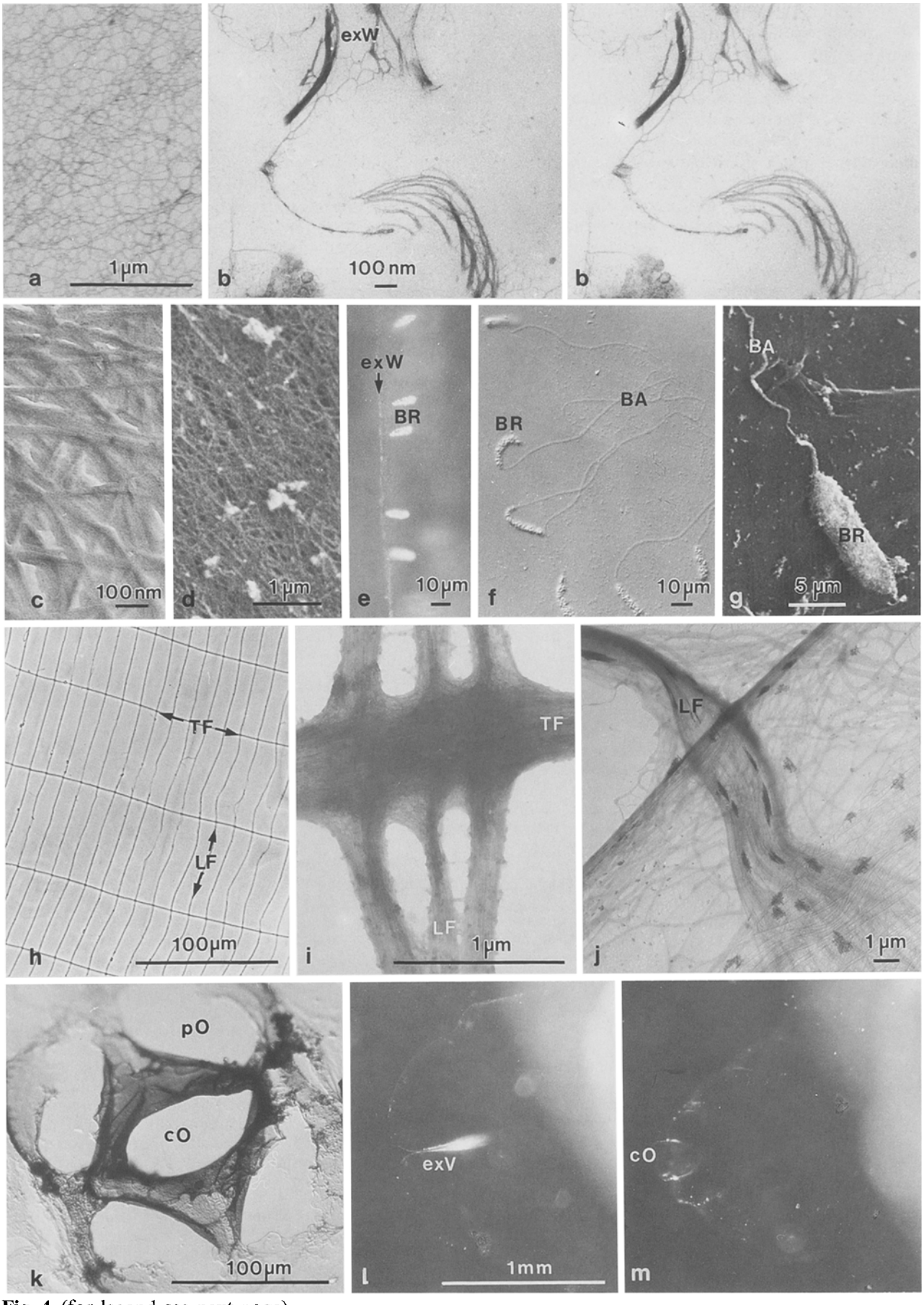


Fig. 4 (for legend see next page)

ness from 5 to 20 nm, and formed pores of 5 to 100 nm width. No membrane-like material or electron-opaque film covering these meshes could be identified, either by stereo TEM (Fig. 4b) or by SEM (Fig. 4d).

Rod-shaped clusters of globular granules, ca. 5×20 µm in size, with slender filaments attached, were present on the extracellular surface of the house (Fig. 4e to g). These rods and "antennulae" are responsible for the bioluminescence associated with *Oikopleura labradoriensis* houses (Galt and Sykes 1983), and are described in more detail elsewhere (Flood and Galt in preparation).

Internal channels and chambers

The bilateral inlet openings led through funnel-shaped passages towards a common midline trunk chamber containing most of the trunk. Near the external orifice of these inlet funnels a coarse-meshed inlet filter traversed the passage (Fig. 3). These bilateral filters displayed very regular rectangular meshes with a length:width ratio of 6 and horizontally oriented longitudinal axes. Pore width was 12.7 ± 2.1 µm (mean $\pm$ SD, $n = 240$) and pore length 74 ± 12 µm ($n = 109$) (Fig. 4h). The longitudinal and transverse mesh fibres were ca. 1.5 and 0.5 µm wide, respectively, and consisted of bundles of filaments, each ca. 10 nm in diameter. At the intersections between the two filamentary bundles, the longitudinal fibres in particular branched out into three sub-bundles on top of, or lateral to, the more compact transverse filamentary bundle (Fig. 4i). At the periphery of the filter, both sets of filaments fanned out into the surrounding walls and intermingled with similar filaments (Fig. 4j).

A large escape chamber was located between the trunk chamber and the bow of the house. This ended in an escape slot towards the exterior along the midline below the bow (Fig. 3A). When the organism was disturbed and left its house this slot was torn wide open and served as an escape passage.

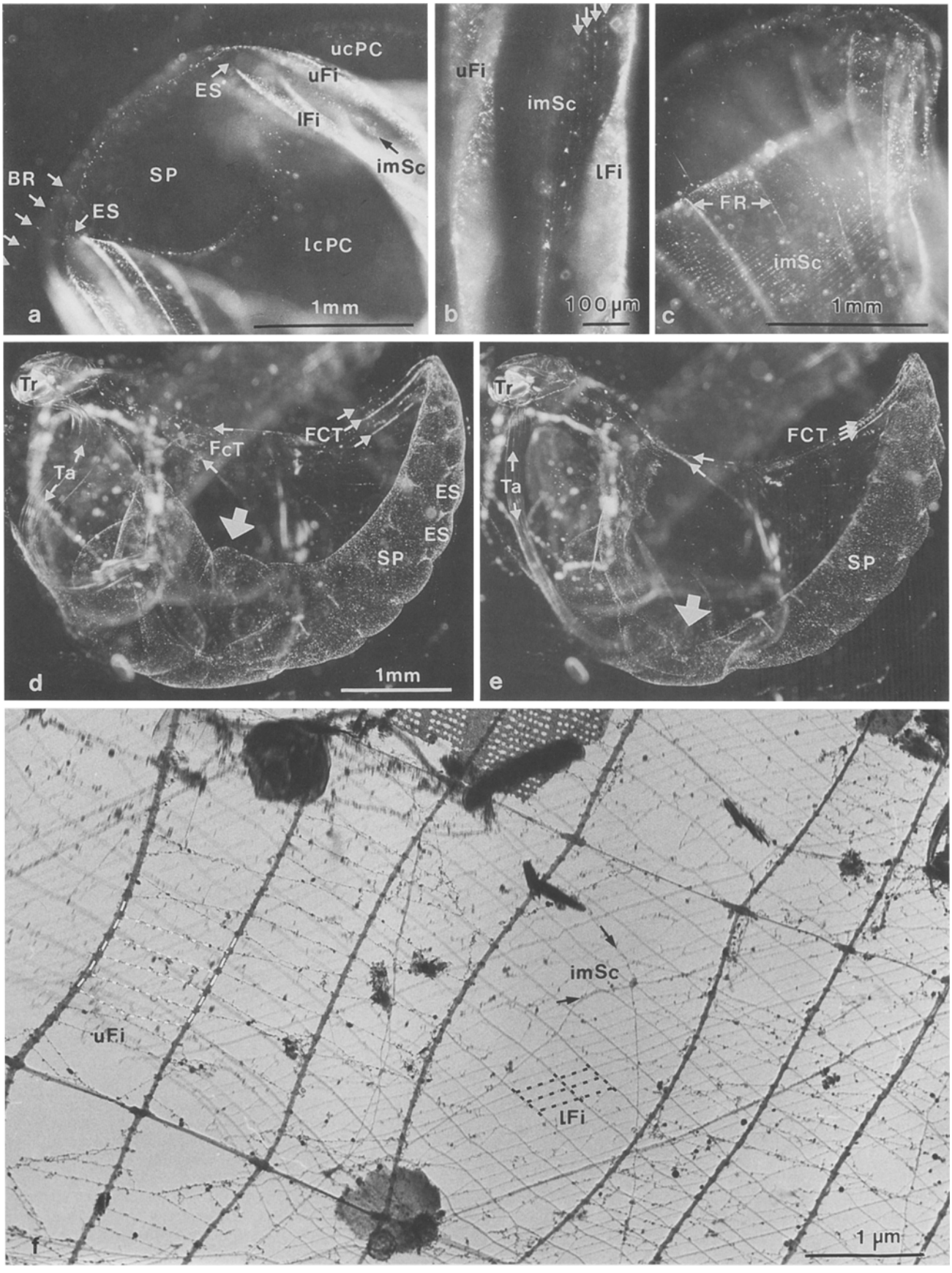
The inlet funnels, anterior part of the tail chamber and bow were surrounded by rather stiff cushion chambers that prevented their collapse. Parts of these cushion chambers produced small outgrowths postero-lateral to the inlet openings (Fig. 3). The stiffness of the cushion chambers seemed to be due to high hydrostatic pressure, rather than to the presence of solid or gelatinized substances within them.

The sagittal trunk chamber continued directly, inferiorly and posteriorly, into a tail chamber, which had a transverse diameter corresponding exactly to the width of the tail and a height just sufficient to provide room for the undulatory movements of the flattened tail. These movements were of greater amplitude at the anterior end of the tail than near its posterior end, so that the height of the tail chamber gradually tapered off towards the tip of the tail. Both the roof and floor of this chamber were prominently cross-striated. According to Deibel and Morris (personal communication) similar striations act like antifriction pads in the house of *Oikopleura vanhoeffeni*. The tail chamber ended abruptly in a curved wall (along the posterior-inferior pole of the house) that separated it from an exit spout (described below). During resting phases, the tip of the tail curled up against the end wall.

Although closed within the sagittal plane, the far end of the tail chamber displayed wide communications, with two lateral passages, one on each side, that led upwards

Fig. 4. *Oikopleura labradoriensis*. Details of the house. (**a**) Transmission electron micrograph (TEM) of total mounted external house wall, stained by Ruthenium Red. (**b**) TEM stereo-pair of 200-nm-thick section of external wall (exW), stained by uranyl acetate and lead citrate. (**c**) TEM of total mounted external wall, shadowed by platinum/carbon. (**d**) Scanning electron microscopy of total mounted external wall, sputter-coated with 10 nm gold/palladium. (**e**) Optical cross-section of external wall, revealing yellow autofluorescence of attached bioluminescent rods (BR) (blue excitation fluorescence light microscopy). (**f**) Differential interference contrast light micrograph (dicLM) of total mounted external wall, revealing granularity of bioluminescent rods and attached filamentous antennulae (BA). (**g**) SEM of bioluminescent rod and antennulae [prepared the same way as in (**d**)]. (**h**) Bright field light micrograph of total mounted inlet filter stained by Toluidine Blue, revealing the extremely regular spacing of the longitudinal fibres (LF) and transverse fibres (TF) of the filter. (**i**) TEM of total mounted inlet filter stained by Ruthenium Red, revealing details of the intersections between longitudinal and transverse fibres. (**j**) TEM of total mounted house, stained with uranyl acetate and lead citrate to reveal the junction between a longitudinal inlet filter fibre and the wall of the inlet funnel. (**k**) dicLM of a microdissected and mounted exit valve stained with Alcian Blue. Note the more prominent staining of the wall of the central opening (cO) than of the peripheral openings (pO). (**l**) Dark field macrograph of a closed exit valve (exV) in lateral view, and (**m**) similar macrograph of the same valve in its half-open position revealing only the central opening

Fig. 5. *Oikopleura labradoriensis*. Details of the food-concentrating trap of its house. (**a**) Dark field macrograph of a supply passage (SP) with adjoining entrance slots (ES) to the triple-layered food-concentrating trap; top projection of an unstained, inhabitated house. The upper filter membrane (uFi), lower membrane (lFi) and intermediary coarse suspensory screen (imSc) are also shown, as well as the lower compartment of the posterior chamber (lcPC) and some bioluminescent rods (BR) attached to the external house wall. (**b**) Detail of the three layers of the food-concentrating trap from the same house as in (**a**). Individual filaments of the intermediary suspensory screen (imSc) are marked by arrows. (**c**) Side view of several filter ridges (FR) and the intermediary suspensory screen (imSc), revealing regularly spaced filaments. Same specimen as in (**a**) and (**b**). (**d**) Dark field macrograph of an actively pumping *O. labradoriensis* in its house. Unstained. Note the trunk (Tr) and curved tail (Ta) of the organism, the expanded supply passage (SP and large arrow), entrance slots (ES), and widely separated layers (small arrows) of the food-concentrating trap (FCT) and food-collecting tube (FcT). (**e**) Same organism and house as in (**d**) but during a resting phase between pumping cycles. Note the conspicuous collapse of the supply passage, food-concentrating trap and food-collecting tube. Same scale as in (**d**). (**f**) Transmission electron micrograph of a microdissected food-concentrating trap of an *O. labradoriensis* house after staining in 1% Ruthenium Red and critical-point drying on a specimen grid without supportive formvar and carbon films. The upper (uFi) and lower (lFi) food-concentrating filters and several fibres of the intermediary suspensory screen (imSc), as well as numerous trapped particles, are superimposed on each other. Fibres and pores of the upper and lower filters are marked by white and black dashed lines, respectively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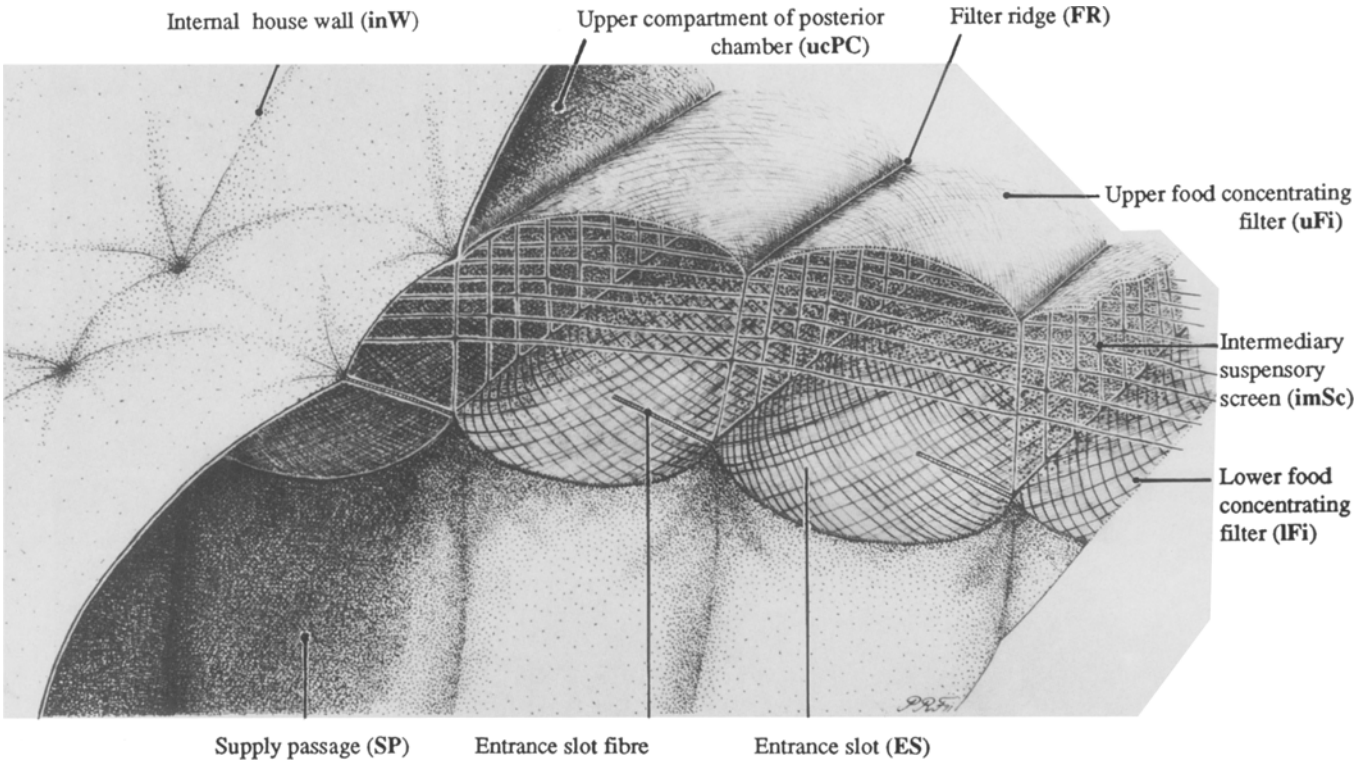


Fig. 6. *Oikopleura labradoriensis*. Sketch illustrating the structural organization of the triple-layered food-concentrating trap and its junction to the supply passage in the house. Note the oblique orientation of the filter screens relative to the direction of the filter ridges. (Note that the filter pores are drawn at a much higher magnification than the ridges)

along the periphery of the house (Figs. 3A, C; 5d, e). These supply passages ended before they reached the superior pole, but each communicated through about 25 apertures or entrance slots, along their anterior, superior and posterior margins, with an extensive particle-filtering or food-concentrating device (Figs. 1; 5a, d, e; 6) that spanned the posterior half of the house as a prominently curved sail (Fig. 3).

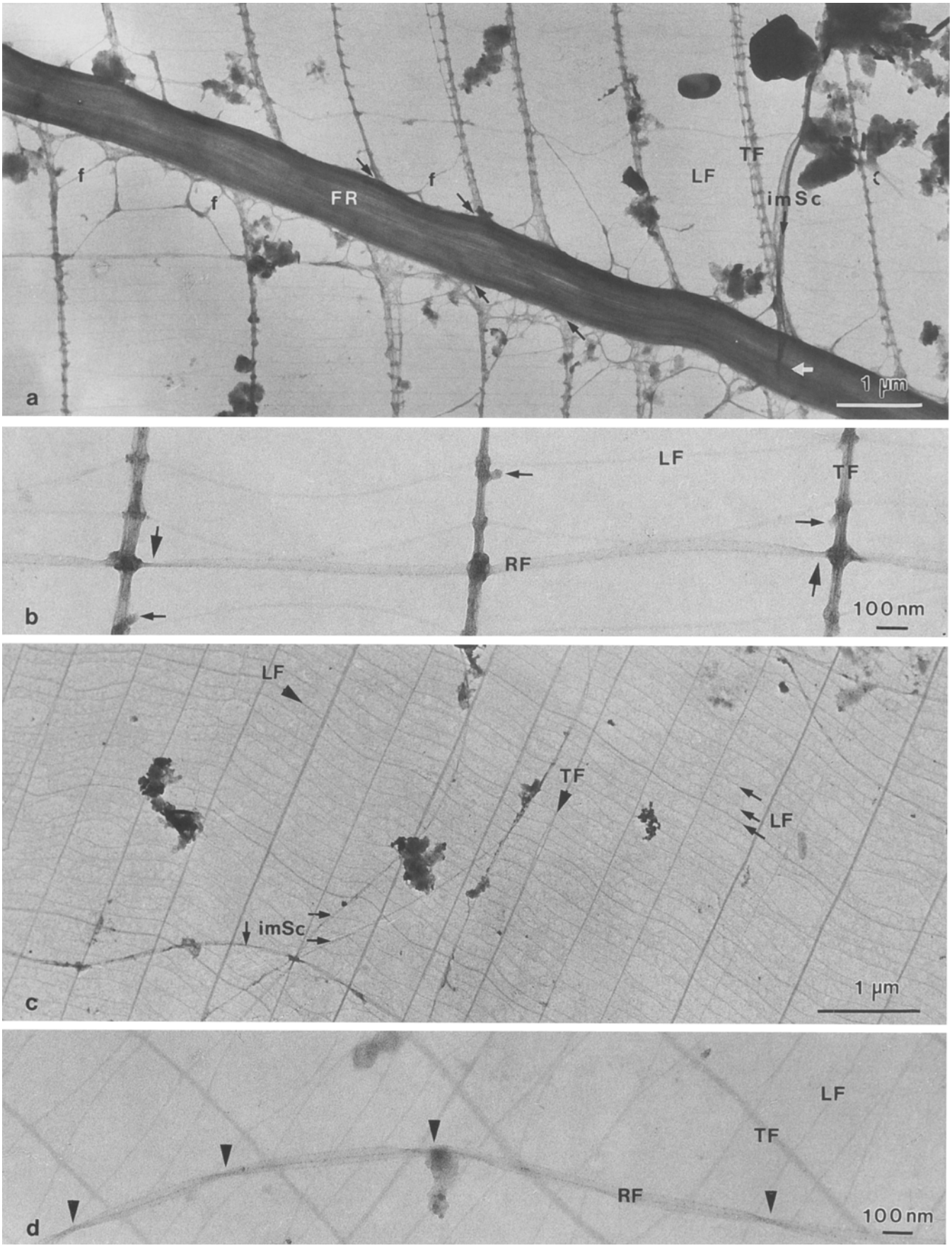
The spaces superior and inferior to this sail-like food-concentrating trap communicated freely with each other behind its posterior border. Anterior to the supply passages, however, the superior and inferior compartments of the posterior chamber seemed to be separated from each other by a non-porous septum. This extended more or less horizontally between the medially situated food-collecting tube (cf. Figs. 3A and 5d, e) and the septum that separated the posterior chamber from the complex cushion chambers and the inlet funnels. Bilateral suspensory filaments extended from this septum to the roof of the house (Fig. 1).

From the inferior-posterior aspect of the large posterior chamber the medial funnel-shaped exit spout ended in an exit valve towards the exterior. In medium-sized houses this exit valve consisted of a 100- to 400-μm-wide, oval to circular central opening and four peripheral oval openings of approximately the same size. A prominent elastic band marked the edge of the central opening (Fig. 4k). When the valve was closed these apertures were hidden in a transverse fold between an upper and a lower membraneous bowl (Fig. 4l). However, these bowls could be everted to expose first the central opening (Fig. 4m) and, at a higher hydrostatic pressure, the four peripheral openings.

The food-concentrating trap

The food-concentrating trap consisted of three layers: an upper and lower filter membrane, held together by a third, intermediary layer of suspensory fibres (Figs. 5 to

Fig. 7. *Oikopleura labradoriensis*. Transmission electron micrograph of microdissected and Ruthenium-Red-stained food-concentrating filters, air-dried on supportive films. **(a)** The lower filter membrane at the level of a filter ridge (FR). Note how the transverse fibres (TF) consist of several filaments and continue across the ridge (black arrows). The attachment of an intermediary screen filament (imSc) in the ridge fibre is also shown (white arrow). Numerous irregular filaments (f) branching from the ridge fibre attach to the filter meshes. (Note: at this magnification, ×15300, the distance between successive ridge fibres is ~7 m). **(b)** Detail of the filter shown in **(a)**. Note the prominent thickening of the transverse fibres (TF) at their junctions with the longitudinal filaments (LF) and their occasional nodules between mesh junctions (small arrows). Some longitudinal filaments are replaced by thicker ribbon-shaped filaments (RF) that stay mostly perpendicular to the screen at their junction with the transverse filaments (large arrows), but fall flat on the supporting film slightly beyond these junctions. **(c)** The upper food-concentrating filter. Note the variable thickness and occasional splitting (arrows) of both the longitudinal (LF) and transverse (TF) fibres, and the lack of thickening at their junctions. Fibres from the intermediary suspensory screen (imSc) are also present. **(d)** Detail of the filter shown in **(c)**, with two adjoined ribbon-shaped filaments (RF) of the intermediary suspensory screen superimposed. Points where the ribbon is parallel to the optical axis are marked by arrowheads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7). Viewed laterally (Fig. 3 C) the trap displayed an inverted U-shape as it originated from the ca. 25 slots along the anterior, superior and posterior edges of the supply passages. These slots also marked the lateral end of parallel filter ridges leading towards the midline (Fig. 3). The filters soon flattened out and merged with those of the contralateral side in a much smoother curve, with their concavities still facing downwards (Fig. 3 C). Near the anterior end of these medially merged filters (merged at the midline), a complex set of flaps or valves marked the transition to a narrow medial food-collecting tube that led anteriorly, directly to the mouth of the animal (Figs. 3 A; 5 d, e). The 12 to 13 most anterior and 4 to 5 most posterior filter ridges ended before reaching the midline (Fig. 3 A to C), and particles trapped between the two sieving membranes here had to pass upwards, perpendicular to the direction of the ridges, to reach the food-collecting tube.

The intermediary layer of suspensory fibres consisted of perpendicular strands which joined corresponding filter ridges of the upper and lower filter membranes together at a constant interval, and parallel strands that formed an intermediary screen halfway between the two filter membranes (Fig. 5 a to c). As the distance between these suspensory fibres was ca. 30 μm – or more than twice the pore width of the inlet filters – the suspensory system did not obstruct the free flow of particles across the ridges. Each fibre was composed of one or several laterally adjoined ribbon-shaped filaments, each ca. 35 nm wide and 10 nm thick. These ribbons had an electron-dense dotted edge and a homogeneous, less electron-dense core (Fig. 7 d).

When viewed from the front the medially merged food-concentrating trap displayed an upright U-shaped profile (Fig. 3 B); the trap was thus rigidly suspended, like a triple-layered sail, in a large posterior chamber that occupied more than half the total volume of the house.

The upper and lower food-concentrating filter membranes were exceedingly delicate, and only their coarser fibers could be discerned by light microscopy (cf. Fig. 3 C in Flood 1991 a). Using TEM a prominent difference in the construction of the two filters became evident (Fig. 5 f), as described below.

The lower filter of the trap (Fig. 7 a, b) exhibited pores 0.24 ± 0.03 μm wide (mean ± SD, $n = 114$) and 1.43 ± 0.17 μm long ($n = 28$). The transverse fibers delineating these pores were made up of 50-nm-thick bundles of 5 to 10 filaments, each ca. 5 nm thick. In some instances, a few filaments left one bundle to join the neighbouring one after having passed through some 20 to 50 intersections. The longitudinal fibers were very regularly spaced and consisted of a single 5-nm-thick filament. However, every 5th to 40th longitudinal fiber was replaced by one or two adjoined ribbon-like filaments (Fig. 7 b), similar to those described above for the suspensory fibres. The transverse fibers were markedly thicker and more heavily stained by ruthenium red at the junctions with both types of longitudinal filament. Similar nodules were sometimes seen projecting from the transverse fibers between successive intersection points (Fig. 7 b) (cf. nodulated fibres in Deibel and Powell 1987 a).

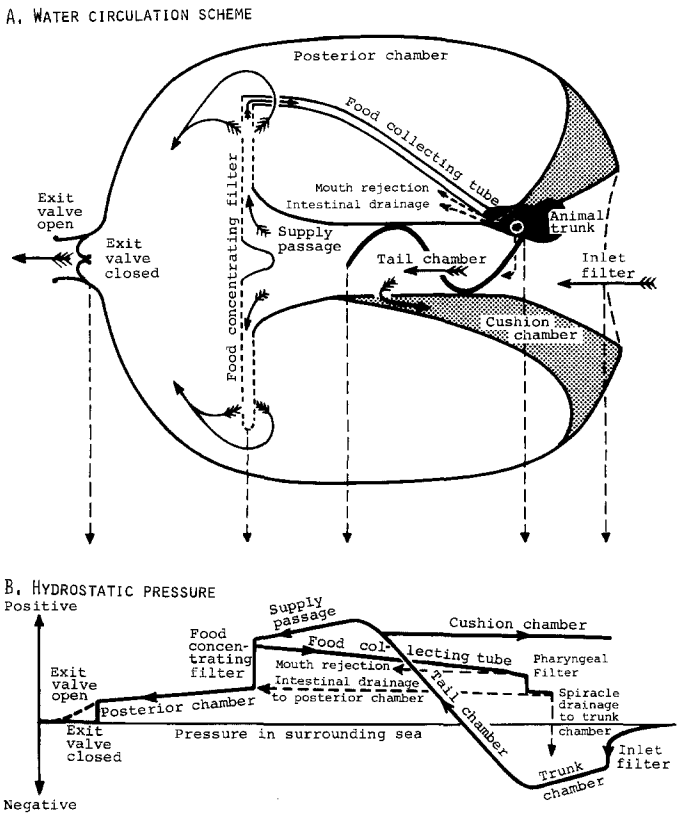

Fig. 8. *Oikopleura labradoriensis*. Schematic diagram of (**a**) water circulation through an idealized oikopleurid house, and (**b**) hypothetical hydrostatic pressure conditions within the same house. (Note: the two inlet filters are displaced forward and replaced by one opening. The shape of the food-concentrating trap is also grossly simplified.) Solid arrows indicate the main flow of water and particles. Dashed arrows indicate alternative rejection currents and the pharyngeal drainage. Note that the cushion chambers are communicating only with the high-pressure end of the tail chamber

The upper filter of the trap stained less prominently than the lower filter (Fig. 5 f) and had pores 0.18 ± 0.03 μm wide (mean ± SD, $n = 98$) and 0.69 ± 0.20 μm long ($n = 66$) (Fig. 7 c, d). The transverse fibers varied in thickness from 10 to 30 nm and apparently consisted of several sub-filaments. Branching of the transverse fibers was more frequently seen in this filter than in the lower one. The longitudinal fibers of the upper filter were also very regularly spaced and usually consisted of a single filament, ca. 5 nm thick. No thickening or altered staining characteristics were seen at the intersections of the longitudinal and transverse fibers in this filter (Fig. 7 d).

The parallel ridges of the food-concentrating trap, described above, were for both the upper and the lower filter membranes composed of 0.6- to 1.0-μm-wide bundles of filaments, each ca. 10 nm thick (Fig. 7 a). The rectangular meshes of the filter membranes usually ran at an angle oblique to the ridges, and particularly in the lower screen it appeared as though the transverse fibers continued across the ridge. Numerous short and irregular thin filaments radiating from the ridge bundle attached to these transverse fibers (Fig. 7 a).

Suspension of the organism within the house

The organism was attached to its house only by its trunk. The oral part of the trunk projected posteriorly through the wall separating the trunk chamber from the upper part of the posterior chamber. Here its mouth was attached to the food-collecting tube (Fig. 5d). The gonadal part of the trunk was lodged in the escape chamber, the anterior wall of this chamber being attached to the posterior margin of the oikoplast epithelium on the dorsal and lateral sides of the trunk (Fig. 3A). Probably the Langerhans bristle receptor (see Bone and Ryan 1979) was embedded in this wall near its inferior margin towards the roof of the trunk chamber. The ventral side of the trunk was also fixed to the house along the posterior margin of the oikoplast epithelium.

Water circulation through the house

The periodic undulatory movements of the organism's tail acted as a pump, sucking water through the inlet funnels into the trunk chamber and forcing it down the tail chamber, out the supply passages and into the food-concentrating trap. Here water was strained through submicrometer pores in the upper and lower filter membranes, and particles and organisms present in the water were retained between the two membranes. The particle-free water that passed through the filters accumulated in the superior and inferior compartment of the posterior chamber, until the hydrostatic pressure there had increased sufficiently to force the exit valve open (Fig. 8a). A jet of water then left the house through the central exit opening and pushed the house a short distance forward through the water.

After a pumping cycle, when the tail stopped beating, the pressure fell and the exit valve closed. The elastic recoil of the thin peripheral walls covering the posterior chamber then forced water back through the food concentrating filters. Particles trapped by the filters were lifted from the filaments of the filter screens and then compacted into coarser aggregates and flakes as the three layers of the trap collapsed completely. During the next cycle the particles and aggregates were brought closer to the midline before they again were trapped in the filters and compacted by the collapsing screens. As the result of several such cycles the aggregates grew considerably in size and were brought all the way to the midline (cf. Fig. 3A).

During reflux periods the supply passages collapsed almost completely (Fig. 5d, e). Their considerable volume of water was forced back through the tail and trunk chambers, causing a pronounced backwashing of the inlet filters.

Particle-enriched water that accumulated between the food-concentrating filter membranes near the midline during the active pumping periods was intermittently passed onward through a complex set of valves before it entered the food-collecting tube, to be transported to the organisms mouth and pharynx. Here the particles were again trapped in a pharyngeal feeding filter, which was almost constantly secreted by the endostyle and moved like a conveyor belt towards the oesophagus and stomach. Even the smallest visible particles entering the mouth of the organism seemed to be trapped in this filter whenever it was present. When secretion of the pharyngeal filter was interrupted, the particles poured out through the spiracles into the trunk chamber. Any particle escaping through the pharyngeal filter, accordingly, was recycled into the food-concentrating trap (Fig. 8a).

Some specimens also occasionally closed their mouth by flipping up the lower lip. The content of the food tube then poured into the inferior compartment of the posterior chamber and left the house through the exit spout. Such a rejection current was seen only when the organism was fed a dense suspension of particles under experimental conditions.

Fecal pellets of a characteristically elongated, ovoid shape, which left the anus, likewise entered the inferior compartment of the posterior chamber and usually escaped from the house through the exit valve (Fig. 8a). However, quite often some fecal pellets failed to be expelled and remained in the posterior chamber, attached to one of its walls (cf. Fig. 9c).

Hydrostatic pressure conditions inside the house

When the oikopleurid is actively pumping water through its house, the hydrostatic pressure in the passages leading to the tail chamber must be lower, and in the passages leading from the tail chamber higher, than in the surrounding sea. Otherwise water will not enter or leave the house through the correct apertures (Fig. 8b). As an obvious result of these hydrostatic conditions the walls of the afferent passages have to be rigid, to prevent collapse, whereas the walls of inflated efferent passages and chambers may be very thin and flexible.

The stiffness of the walls of afferent passages seemed to depend on high hydrostatic pressure in the cushion chambers. This pressure was apparently derived from the communication of these chambers with the posterior-inferior end of the tail chamber. Alternatively, their high pressure could have been due to wide communications with the upper compartment of the posterior chamber.

When the organism stopped beating its tail, no pressure was generated, and the water was able to rush back from the high- to the low-pressure chambers. Due to rapid closure of the pressure-regulated exit valve and elastic recoil of the walls of the posterior chamber, a reflux of water occurred, causing a transient collapse of the food-concentrating trap (Fig. 5e) and a pronounced backwashing of the inlet filters.

Speed and flow rate of water through the house

During the equilibrium state, when the organism is actively pumping water through its house and the exit valve remains open, water flow through the various passages must be the same, irrespective of their diameter. Accordingly, the amount of water passing through the house per unit time may be calculated from data on water speed through a single passage of known cross-sectional area.

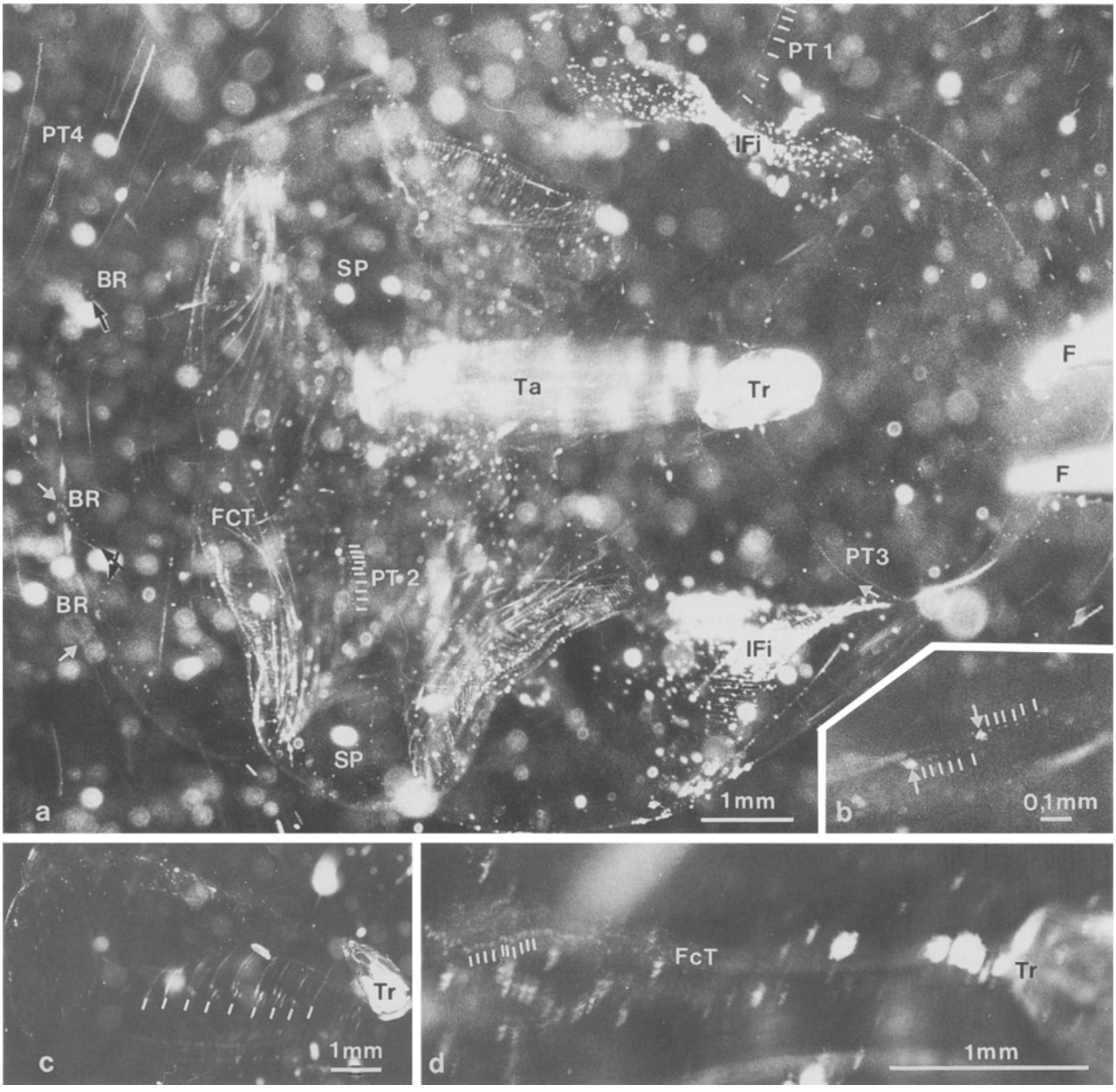

Fig. 9. *Oikopleura labradoriensis.* In vivo strobe-flash dark field micrographs of inhabitated house ca. 1.1 cm in length, at ca. 11 °C (organism trunk length 1.2 mm). **(a)** Entire house held in position by forceps (F) and exposed for ~1 s at 1000 flashes min^{-1}. Linear particle tracks, indicating laminar flow, are seen towards the inlet filter (PT1), through the supply passage (PT2), through the inlet funnel (PT3), and outside the house towards the exit spout (PT4). Note also the presence of linear arrays of bioluminescent rods (BR). Other abbreviations as in Fig. 1. **(b)** Similar picture, showing two particle tracks that end by trapping of the particles (arrows) in the food-concentrating filter (1000 flashes min^{-1}). Due to slight movement of the filter the trapped particles are imaged as a cluster of dots. **(c)** Similar picture of the tail wave-front movement within the tail chamber (1000 flashes min^{-1}). **(d)** Similar picture of a particle moving through the food-collecting tube (FcT) (600 flashes min^{-1}). Due to vibrations of the actively pumping animal, the track is slightly zig-zag shaped

For medium-sized oikopleurids and houses with tail chambers 1 × 2 mm in cross-section, subjectively selected for normal pumping behaviour, strobe-flash micrographs of the tail movement in the close-fitting tail chamber indicated a flow rate of ~0.84 ml min^{-1} (median value for five specimens at a water temperature of 11 to 12 °C) (Fig. 9c). Taking the intermittent pumping pattern (Fig. 10) into account, this corresponds to ~35 ml h^{-1}.

Strobe-flash micrographs also indicated that particles entered the inlet filters with speeds of up to 5 mm s^{-1} (Fig. 9a, Particle Track 1), the supply passages at a speed of ~1 to 2 mm s^{-1} (Fig. 9a, Particle Track 2), and the food-concentrating trap at a speed of ~0.4 mm s^{-1} (Fig. 9b). Roughly estimated, the total area of upper and lower food-concentrating filters was close to 100 mm^2. This indicated that water seeped through these filters at

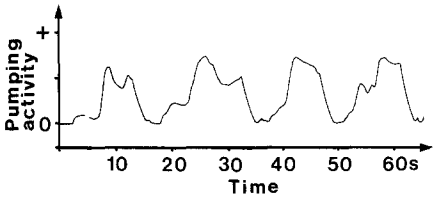

Fig. 10. *Oikopleura labradoriensis.* Recording from an inhabited house of the cooling effect of the exit jet of water on a heated thermocouple held <2 mm behind the exit spout. The temperature curve reflects the intermittent and variable pumping speed of the organism

a speed of ~2.5 μm s^{-1}. Accordingly, the current speed was much higher between (parallel to) the filters than across them. Such tangential flow filtration caused particles to be transported towards the midline of the trap.

The particle concentration factor

Particles that approached the organisms mouth through the food-collecting tube were measured as travelling at a speed of ~0.3 mm s^{-1} (Fig. 9d). For intermittent and laminar flow through a food tube of 250 μm diameter and circular cross-section, this corresponds to a water flow of ~0.04 ml^3 h^{-1}. This means that the organism, by aid of its house, is likely to feed on a suspension of particles that is ~900× concentrated relative to the particle density in the surrounding sea.

Propulsion of the house through the sea

For a circular exit-valve opening of 0.4 mm diameter, which was usual for steady-state, medium-sized houses, the exit jet should have a speed of ~120 mm s^{-1}. This was sufficient to force the entire house forward through the sea at a speed of ~1 cm s^{-1}. For oikopleurids pumping at normal rates the course of the house was linear and usually horizontal, the natural sinking tendency of the house and organism being just compensated by the upward push caused by the upward-pointing bow and the position and direction of the exit spout below the house's center of gravity. However, for very actively pumping organisms the upward push increased, and a somersaulting movement, with loops ca. 10 cm wide, was seen (cf. Alldredge 1976b). Due to the inertia of the water contained in the house, its forward movement also continued during the intervals between pumping cycles. This, together with the blunt cushion-chamber flaps postero-lateral to the inlet openings, prevented backwashed particles detached from the inlet filters from being sucked back. In turbid tidal water, therefore, some houses were observed to leave behind a bilateral track of coarse particle aggregates, resembling the wake of a miniature rowboat.

Pumping pattern of the organism

Undisturbed oikopleurids observed in the field pumped water intermittently through their houses. To measure the duration of pumping periods, a heated thermocouple was held behind the exit spout of inhabitated *Oikopleura labradoriensis* houses, clamped in position in a deep Petri dish by forceps. The cooling effect of the exit jet of water revealed pumping cycles of 7 to 14 s duration, separated by ~5-s intervals in most cases. A slow and fast pumping rate, corresponding to distinct tail-beating frequencies, could usually be discerned within each cycle (Fig. 10).

Discussion

General architecture of the house

In spite of evident differences in house architecture among distinct oikopleurid species (Alldredge 1977, Flood 1983, Deibel 1986, Fenaux 1986, Flood et al. 1990), there are also pronounced similarities. Except for the lack of inlet filters in *Oikopleura longicauda*, all the internal filters, chambers and passageways seem to have been present in all epipelagic species examined so far.

To the extent that morphological differences do occur, these relate to the shape of specific recesses in the peripheral part of the internal walls and in the separation between the external and internal walls. *Oikopleura labradoriensis* houses have no "keel" and very small "sails" postero-lateral to the inlet funnels. The comparable structures are highly developed in *O. vanhoeffeni* (Flood et al. 1990), but absent in *O. dioica* [Fenaux 1986, Flood 1983, 1991b (Fig. 1)]. In *O. labradoriensis* the external walls are less than 1 mm away from the internal chambers except at the bow, where this interval increases to about 2 mm. In *O. vanhoeffeni* houses, ca. 7 cm in length, the comparable values are ca. 3 and 9 mm (Flood et al. 1990, Flood 1991a). In the giant oikopleurid houses recently discovered at mesopelagic depths in the underwater Monterey canyon, the comparable separation between external and internal walls may be several feet, as judged from the video frame shown by Robison (in Gore 1990, p. 31; see also Barham 1979). The increased external house diameter relative to organism size may prevent larger and heavier oikopleurids from sinking more rapidly in the sea.

Water circulation through the house

Lohmann (1899, 1933/1956) identified a closing (Verschluss) membrane that fell back on the internal surface of the inlet filter of *Oikopleura dioica* when the organism stopped beating its tail, preventing any backwashing of the filter. No other author, including me, has seen such a membrane, and the inlet filters are evidently backwashed and freed from adhering particles in all the species I have examined (*O. dioica*, *O. vanhoeffeni*, *O. labradoriensis*, *O. albicans*).

Fenaux (1986) described, in *Oikopleura dioica*, a membrane and flap that covered the external inlet openings. These flaps were inverted or everted according to the inward or outward flow of water through the house. I have been unable to observe such mobile flaps in *O.*

labradoriensis; only a few thin filaments spanning the gap across the inlet funnels moved slightly in and out, according to the direction of water flow.

Concerning the entrance of water to the food-concentrating trap, Lohmann (1899, 1933/1956) evidently was aware of the medial walls of the supply passages (his "Zwischen-Flügel-Kammer" or between-wing-chamber), and he knew that water entered the food-concentrating trap through the lateral entrance slots curved around the edge of the supply passages. The same view was held by Körner (1952), Jørgensen (1966) and Fenaux (1986). Alldredge (1977) and Deibel (1986), on the other hand, concluded that water flowed into the food-concentrating trap both through this route and along the edge of the most anterior and posterior filter ridges. I support the latter view, which means that the supply passages, before reaching the entrance slots, must have a medial wall at their bases that joins the most anterior and posterior filter ridges, leaving an open entrance into those filter ridges for the water.

In *Oikopleura labradoriensis* water escapes from the food-concentrating trap through both the upper and lower filter membrane to fill the upper and lower compartments of the posterior chamber. This was also found by Deibel (1986) for *O. vanhoeffeni* houses. From these compartments water is free to leave the house through the exit spout and valve, provided that the hydrostatic pressure in the posterior chamber is high enough to force this valve open.

The presence of an evertible or erectile exit spout and valve has already been noted by Lohmann (1899), who also commented on its elastic properties. However, he supposed the exit spout to be connected with the tail chamber and that the organism diverted part of the pumped water directly through this valve for propulsion purposes. He also stated that the tail, when resting, blocked the passage from the tail chamber to the exit spout. Alldredge (1977) supposed that the exit spout was directly connected to the midline junction of the two food-concentrating filters. Fenaux (1986) described one large central and four smaller peripheral openings in the exit spout, the latter ones open when the tail beats slowly, and the former one open only when the tail beats strongly.

Except for the presence of one central and four peripheral openings, I find none of these statements valid for *Oikopleura labradoriensis*. The exit spout is connected to the posterior chamber and drains water from both its upper and lower compartment. Further, I find (at least on *O. labradoriensis* and *O. vanhoeffeni*) that the central large opening opens at a lower hydrostatic pressure than do the smaller peripheral openings (cf. Flood et al. 1990). This sequence seems also to be more economical with respect to generation of propulsive force. The hydrodynamic purpose for the addition of four openings, draining water sideways, may be to prevent a long exit spout from spinning like an unsecured waterhose. If the main jet deviates from its straight-backwards orientation, the lateral slits on the convex side of the spout will open more than those on the concave side. The increased water jet which this causes to one side may tend to correct the orientation of the entire house until the main jet again points directly backwards.

The food-concentrating trap and food ingestion

Most of the controversies in the literature concerning the passage of water through the food-concentrating trap are due to a lack of precise knowledge about the construction of this exceedingly delicate structure.

A triple-layer structure of the trap has previously been found by Lohmann (1899, 1933/1956), Körner (1952) and Jørgensen (1966). All these authors supposed a flow of water parallel to the layers – towards the midline between the lower and intermediate layer, and towards the lateral border between the intermediate and upper layer. Their hypothesis supposes the straining of particles to take place mainly at the medial border, where the water should be shifted from the lower to the upper passage between the layers. This would leave the house with a very small filter area that would rapidly clog up.

Alldredge (1977) supposed the exit spout of the house to be directly connected to the midline junction of the food-concentrating trap and ascribed no function to the large posterior chamber. Observing just two layers in the filter, but realizing the possibilty of a third layer, she advanced two hypotheses concerning the straining of particles in the food-concentrating filters, neither of which I find valid for *Oikopleura labradoriensis* houses.

Fenaux (1986), studying *Oikopleura dioica*, thought the food-concentrating filter consisted of two layers and that water escaped only through the lower of these. Nor did he see a function for the upper compartment of the posterior chamber (his "dorsal chamber"), but reported it to communicate with the lower compartment of the posterior chamber (his "exit chamber") by three routes. I am able to verify the presence of just one of these, namely the wide communication between the two compartments, posterior to the food-concentrating filter. I never saw opening and closing valves between these compartments lateral to the food-collecting or buccal tube, nor could I observe any communication between the upper compartment of the posterior chamber and the trunk chamber (his "anterior chamber"). Fenaux (1986) did not see the latter opening, but inferred its presence from fecal pellets sometimes found in the trunk chamber. He ascribed a function of hydrostatic pressure equilibration to this opening. However, pressure must be high in the posterior chamber in order to open the exit valve and produce the jet that propels the house through water, and must be lowest in the trunk chamber so as to suck water into the house (Fig. 8 b). In my opinion, the equilibration of pressure proposed by Fenaux (1986) is incompatible with the function of the house.

As described by Flood (1978, 1981, 1983), Deibel (1986) and Deibel and Powell (1987 a), and as illustrated in the present work, the food-concentrating trap consists of two exceedingly fine filter membranes held together at a specific distance by an elaborate meshwork of filaments that partially compose a definite third and intermediary layer in the food-concentrating trap. This intermediary

layer has, however, a gap between its filaments that is more than twice the pore size of the inlet filters. Accordingly, it has no sifting function.

Contrary to previous beliefs, the construction of the the upper and lower food-concentrating filters of *Oikopleura labradoriensis* are different, probably reflecting their origin from distinct cell groups of the oikoblast epithelium. Similar differences are also present between the upper and lower food-concentrating filters of *O. albicans, O. vanhoeffeni* and *O. dioica* (own unpublished observations). There is also a marked difference in the structural appearance and staining properties of their constituent filaments, implying chemical differences at the molecular level. Further, at least in *O. labradoriensis*, a statistically significant difference is present in both pore width and pore length. So far, it is not known whether the convex-concave geometrical shape of the trap, relative to its hydrostatic expansion and the oblique orientation of the rectangular meshes, tends to augment or counteract this difference in pore size. At present, it seems unrealistic to expect a sharp and well-defined cutoff value for particle sizes strained through the oikopleurid food-concentrating traps.

The intermittent pumping activity of the tail and the elastic recoil of the walls of the large posterior chamber seem to be highly beneficial for the functioning of the house, for two reasons. First of all, the frequent backwashing of filters prevents rapid clogging of their pores, thus prolonging the useful life span of each house and saving the organism energy for both pumping and the synthesis of new houses. Secondly, the frequent collapse of the food-concentrating trap causes pronounced particle aggregation or coagulation, thereby facilitating recapture of food particles in the organism's pharynx. The passage of a highly concentrated suspension of food particles past complex flaps or valves at the transition between the food-concentrating filter and the food-collecting tube, and the passage of the dense suspension along this tube, may possibly cause additional aggregation. Hereby, particles in true colloidal solution, probably ranging in size from <0.2 µm to >1 µm (Hemenz 1986, Kepkay and Johnson 1988, Isao et al. 1990), may be trapped and converted to sizeable food particles for oikopleurids. In fact there is new experimental evidence that at least *Oikopleura vanhoeffeni* is capable of utilizing true colloidal particles of <0.2 µm diameter as food (Flood et al. submitted).

The presence of rejection currents of particle-rich water at the attachment site of the food-collecting tube around the organism's mouth has been noticed by both Deibel (1986) and Fenaux (1986). In their opinion the rejection stream is bilateral and originates at the dorsal lip, whereas my observations indicate that the lower lip may flip up for short periods so to close the mouth and leave a hole in the floor of the food-collecting tube. High-resolution observations seem to be needed in order to clarify this detail.

According to Deibel and Powell (1987b) the pore width of the pharyngeal filter of *Oikopleura vanhoeffeni* is ca. 3 µm. This is ca. five and ten times, respectively, the mesh size of the homologous pharyngeal filters of salps (Bone et al. 1991) and ascidians (Flood and Fiala-Medioni 1979, 1981). Still, such a coarse mesh size, compared to the 0.2 µm of the external food-concentrating filters, may make hydrodynamic sense, since extensive particle aggregation takes place in the external food-concentrating trap. Particles passing through the pharyngeal filter are also recirculated to the external food-concentrating trap for further coagulation until they are large enough for trapping in a coarse-meshed pharyngeal filter.

Speed and flow rate of water through the house

The speed of water through the narrow passages of the *Oikopleura labradoriensis* house never exceeded 10 mm s^{-1}, except through the exit spout, where a speed of 120 mm s^{-1} was calculated. Taking into account the minute size of the passages, the Reynold numbers therefore always remained orders of magnitude below values at which turbulent flow might be expected (Vogel 1981). A laminar flow of water around the house, throughout its internal passages and through its exit jet is in full agreement with all my observations.

Alldredge (1977) examined water flow through the houses of six oikopleurid species by timing the transit time of dye entering and leaving houses in the field, and by measuring (based on photographs) mean volume of house passageways. She arrived at values varying between 36 and 1477 ml h^{-1}, depending on the size of species and individuals. However, her values apparently suppose the water to be replaced at an equivalent rate from all chambers of the house and do not correct for the frequent periods of non-pumping and of backwashing of the filters. In spite of the overestimate this may lead to, the values given by Alldredge (1977) are in fair agreement with my estimate of ca. 40 ml h^{-1} in *Oikopleura labradoriensis* houses of medium size.

Presuming that oikopleurid houses filter water with close to 100% efficiency for the type of particles used in clearance experiments, the flow rate discussed above may be compared with clearance rates. Such rates are given by Paffenhöfer (1976), Gorsky (1980), King et al. (1980), Alldredge (1981) and Fenaux and Malara (1990) for *Oikopleura dioica*; these authors arrived at values near 8, 2, 4, 12 and 1 to 9 ml h^{-1} respectively for this species, which is definitely smaller than *O. labradoriensis*. Alldredge (1981) also studied *Stegosoma magnum*, a warm-water species larger than *O. labradoriensis*, and found a mean clearance rate of 100 ml h^{-1}. Deibel (1988), who studied the cold-water species *O. vanhoeffeni*, presented an equation from which a flow rate of 17 ml h^{-1} may be calculated for a specimen comparable to a medium-sized *O. labradoriensis*. Knoechel and Flynn (1989) arrived at ca. 700 ml d^{-1}, or 30 ml h^{-1}, for *O. vanhoeffeni* of ca. 9 mm length (which is larger than the size of the specimens I have examined).

Although temperature probably has a pronounced effect on tail beating frequency and thereby on pumping rate, it seems to me as if these clearance rates are low compared to the flow rate estimated for *Oikopleura labradoriensis*. This may be explained by a $<100\%$ efficiency of the oikopleurid house as a particle trap; the rejec-

tion of some strained particles before they enter the mouth (Deibel 1986, Fenaux 1986, present study) may account for such reduced efficiency.

The particle concentration factor

The oikopleurid house functions as a trap to concentrate particles before they are ingested. The most important functional parameter of the house, in addition to the volume of water that is filtered through it and the pore size of its filters, is therefore its particle concentration factor. This may be defined as the particle density in the water entering the organism's mouth relative to the particle density in the open sea.

Lohmann (1933/1956) stated that the concentration factor was most certainly more than 50×; Jørgensen (1984) mentions 100×. I find it much more likely that *Oikopleura labradoriensis* feeds on a close to 1000-fold concentrated suspension of particles. This is in fair agreement with values obtained for *O. vanhoeffeni* houses by Deibel and Morris (in preparation).

The energy cost of pumping

Both pore size and filtration speed through the food-concentrating filters of *Oikopleura labradoriensis* houses are comparable to those of ascidian pharyngeal filters (Flood and Fiala-Medioni 1979, 1981, Flood 1981, 1982). Accordingly, one may also expect the pressure gradient across the filters and the energy spent for filtration to be comparable. Based on measurements made on *Styela clava*, Riisgård (1988) found energy consumption to equal 0.04 $\mu W\ min^{-1}$ for a pumping rate of 0.6 $ml\ min^{-1}$ (which is likely for a large *O. dioica* house). This value corresponds to <2% of the total oxygen consumption of *O. dioica* as determined by Ikeda (1974) and Gorsky et al. (1987). Therefore, even if severely underestimated, it is unlikely that the sieving of particles from the water constitutes a major energy cost for the organism. The energy required for pumping water through the channels of the house, and especially through the exit spout to propel the organism and house through the sea, may be greater than that spent for filtration alone.

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