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Morphological and histochemical examination of male and female gonads in *Homarus gammarus* (L. 1758)

Research Article

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Abstract: The reproductive organs of both male and female European lobsters (*Homarus gammarus*) are H-shaped gonads that lie dorsal to the gut on the large hepatopancreas. The ovary consists of a pair of tubular, parallel lobules with a connecting bridge. The germarium of the ovary containing oogonia is concentrated in the center of the ovarian lobe. As oogonesis proceeds, the oocytes move to the peripheral regions of the ovary. The follicle cells begin to surround the oocytes in the previtellogenic stage, and the mature oocytes are completely surrounded by the follicle cells. Carbohydrates exist in both early and late vitellogenic oocytes that give PAS positive reaction. However, their rising protein content in late vitellogenic oocytes makes them stain with Bromophenol blue. Testes show convoluted lobules with a germinal epithelium and a central collecting duct, and the paired vasa deferentia have three distinct parts. Spermatophores are nonpedunculate and tubular, which extrude as a continuous column and consist of a sperm mass covered with primary and secondary layers. The primary layer stains with Bromophenol Blue and gives a PAS positive reaction. But the secondary layer only weakly stains with Bromophenol Blue. The histochemical results may indicate that the function of the two layers is different.

Keywords: *Homaridae • Reproductive biology • Ovary • Testis*

1. Introduction

The European lobster (*Homarus gammarus*) has a geographical distribution that starts from the Lofoten islands of North Norway and the south-west coast of Sweden and Denmark in the north (except the Baltic Sea), south through the coasts of Britain and Ireland, ending in the south at the Atlantic coast of Morocca. This species also is widely distributed throughout the Mediterranean Sea, among the Mediterranean islands and east to the point where the Dardanelles meet the Aegean Sea [1,2]. *H. gammarus* is an endangered species in Helgoland [3] and has high commercial value as food [3,4]. Overfishing, habitat destruction, increasing sea tempratures and increased industrial pollution cause difficulties for the reproduction of *H. gammarus* and its natural stocks are harmed accordingly [5]. For these reasons, like other important sea products, the management of *H. gammarus* has become vitally important [6,7]. Published studies on reproduction of *H. gammarus* are very limited, but are important to protecting natural stocks and to solving problems regarding their reproduction. Hence, basic studies on the structure of gonads and reproduction of this species need to be performed, to increase this body of knowledge.

Various studies have investigated reproduction in *H. gammarus*. These include: growth, reproduction, movement and abundance of the European lobster, *H. gammarus,* at the rocky island of Helgoland, North Sea [8], improvement of rearing conditions for juvenile lobsters by co-culturing with juvenile isopods (*Idotea emarginata*) [3], growth, reproductive cycle, and movement of berried European lobsters in a local stock off southwestern Norway [9], reproduction in the European Lobster [10], studies on the developmental conditions of the European lobster [8], and variation on size at onset of egg production [11]. However, basic studies concerning the male and female reproductive system, gonad structure and histochemistry are missing.

The aim of this study is to describe the female and male reproductive system of *H. gammarus* in detail,

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analyse the ovary, oviduct, testis and vas deferens morphologically and histologically, the development of oocytes and spermatophore histochemically and to compare these features within other Astacidea.

2. Experimental Procedures

Eight adult male (mean body length: 25.8 cm, mean body weight: 700 g) and female (mean body length: 26 cm, mean body weight: 730 g) specimens of *H. gammarus* were collected from Dardanelles, Turkey. The animals were killed by cold treatment (5 min., -20ºC). Lifting the dorsal carapace, the testis, the vasa deferentia, the ovary and the oviducts were dissected and fixed with Bouin's solution for 12 h. After dehydration through an ethanol and xylene series, samples were embedded in paraffin wax. Paraffin sections were cut at 5 µm thickness and stained with hematoxylin-eosin (H+E), Masson's Trichrome and Bromophenol blue were used as a color marker for protein. Also the Periodic acid–Schiff (PAS) and Alcian blue tests for histochemical analysis under light microscopy [12,13] were performed.

3. Results

The female reproductive system of *Homarus gammarus* is composed of the ovary and oviducts. The H-shaped mature ovary is dark green in color, located dorsally in the cephalothorax and lies on the hepatopancreas posteriorly until the fifth abdominal segment. The H-shaped ovary consists of a pair of tubular, parallel lobules with a connecting bridge (Figure 1). Mean total length of the ovary is 14 cm (mean carapace length 11 cm and mean body length 26.1 cm). In the middle of each ovarian lobule, a narrow oviduct protrudes laterally, leading straight to the genital pore on the coxa of each third walking leg (or P3).

The outermost layer of ovary consists of a thin epithelium and muscle fibers, with connective tissue and blood vessels beneath each, respectively (Figure 2). The outermost epithelium and muscle fibers also continue throughout the oviduct and surround it (Figure 3A).The innermost layer of the oviduct is an epithelium composed of a monolayer of columnar cells which produces one type of secretion which does not stain with Bromophenol blue or Alcian blue but does give a PAS positive reaction (Figure 3B).

The germarium, which is the proliferative zone containing mainly oogonia, is located all along the center of both ovarian lobules as a germinal cluster (Figure 4A). In the germarium, only a couple of follicle cells begin to surround the oogonia. As they proceed into the previtellogenic stage, the number of follicle cells increases. When the oocytes reach the vitellogenic stage, they are completely enclosed by a single layer

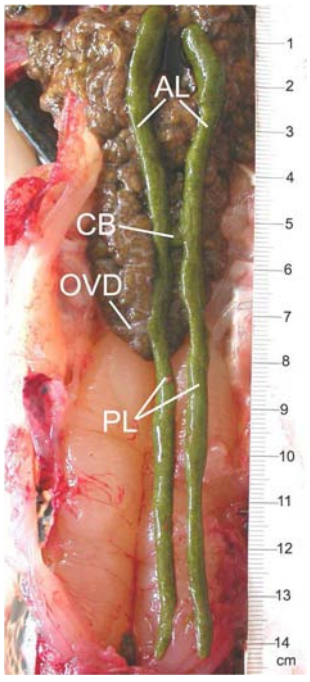

Figure 1. Dorsal view of female reproductive system in *Homarus gammarus*. Two parallel lobed ovaries are composed of anterior lobules (AL) and posterior lobules (PL) joined at the central bridge (CB). In the middle of each ovary, between the two ovarian lobules, a narrow oviduct (OVD) protrudes laterally and leads to the female gonopore.

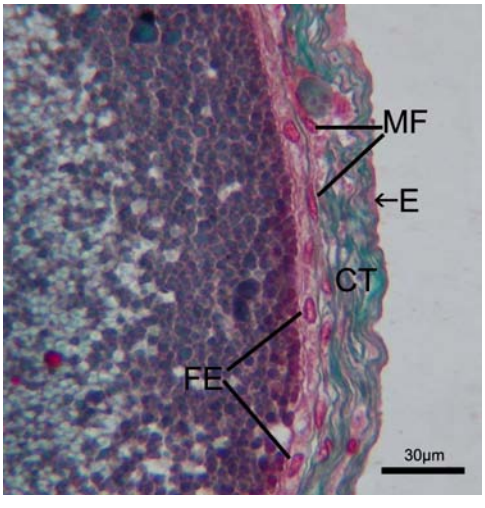

Figure 2. Transverse section showing the layer which surrounds the ovary wall of *Homarus gammarus*. The outermost layer of the ovary consists of a single layer of thin epithelium (E), with muscle fiber (MF) and connective tissue (CT) under it. Collagen fibers are visible in the connective tissue and beneath them lies a single layer of follicle epithelium (FE), which stains with Masson's Trichrome.

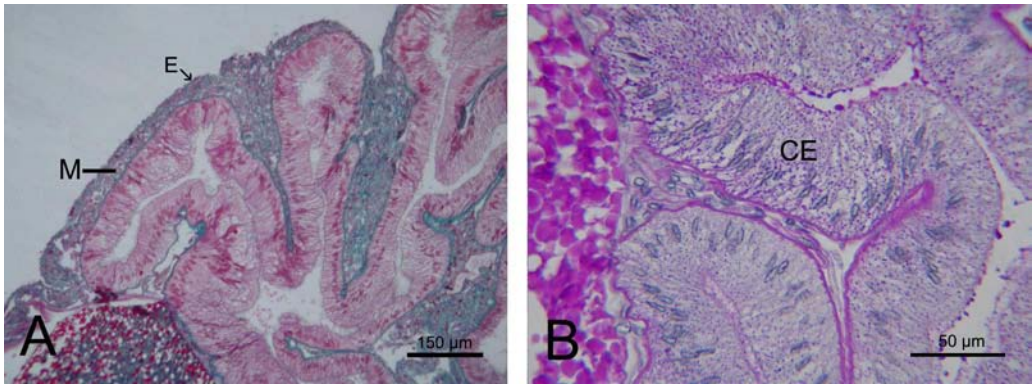

Figure 3. Histochemical structure of the oviduct of *Homarus gammarus*. A. Transverse section through the wall of oviduct showing a single layer of thin epithelium (E) and muscle bundles (MF) which stain with Masson's Trichrome. B. Innermost layer of the oviduct is composed of an epithelium composed of a monolayer of columnar cells (CE), which gives a PAS positive reaction.

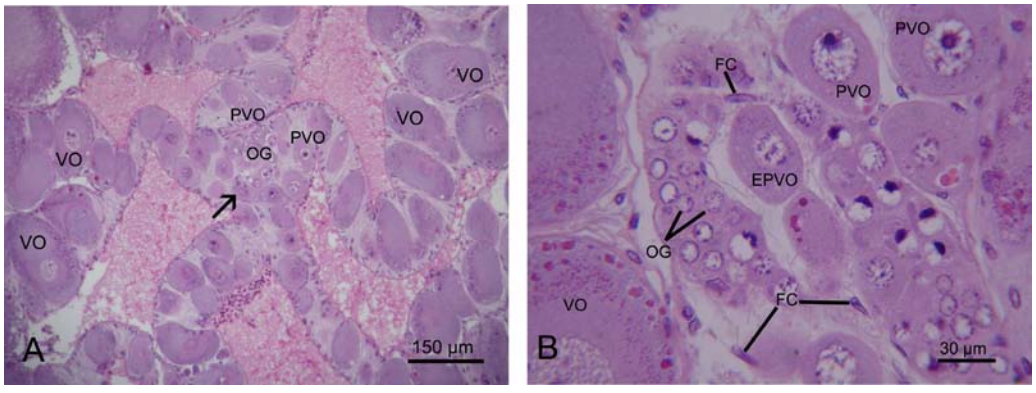

Figure 4. General view of the ovary of *Homarus gammarus*. A. Germinal cluster (arrow) seen radially in the center of the ovary in proliferation stage. Oogonia (OG) and previtellogenic oocytes (PVO) are located in the central region, while vitellogenic oocytes (VO) are located in the periphery. B. The ovary is composed of oogonia (OG), follicle cells (FC), early previtellogenic oocytes (EPVO), previtellogenic oocytes (PVO) and vitellogenic oocytes (VO).

of follicle cells and move to the periphery of the ovary. The oogonia, which are generative cells, are 10-15 µm in diameter. The membrane of the oogonium is not well defined; however, the nucleus, which is located in the center and contains basophilic chromatin material, has a well-defined membrane. At this stage thread-like chromatin proceeds into granular chromatin in the nucleus of the oogonia and the cytoplasm is weakly eosinophilic. While most of the basophilic follicle cells are scattered throughout the acini, only some of them begin to surround the oogonia (Figure 4B).

Previtellogenic oocytes are concentrated as a central germinal cluster in the ovary. Early previtellogenic oocytes (EPVOs) (20-31 µm in diameter) and previtellogenic oocytes (PVOs) (30-60 µm in diameter) arrange radially around the germarium, as the EPVOs and PVOs are located near the germarium and adjacent to the vitellogenic oocytes (VOs) (Figure 4A). The follicle cells begin to surround the PVOs and then form a single layer around the VOs. Both the EPVOs and PVOs have a large, round and centrally located nucleus containing granular chromatin material. The accumulation of PAS positive material at the periphery of the cytoplasm can be observed in previtellogenic oocytes at this stage (Figure 4B).

Vitellogenic oocytes (VOs) are 70-330 µm in diameter at this stage with a round, centrally located nucleus. Generally the vitellogenic oocytes that are larger than 187 µm in diameter are completely surrounded with their own follicle epithelium. Fine yolk granules first appear in the cytoplasm of the earliest vitellogenic oocytes. The size and number of yolk granules increase and they fill the ooplasm except for the perinuclear zone. These yolk granules are PAS positive and also weakly stain with Bromophenol blue, but do not stain with Alcian Blue (Figure 5A,B). A number of small lipid droplets, which are PAS negative and do not stain with H+E, Bromophenol blue and Alcian blue, occur in the ooplasm of the vitellogenic oocytes for the first time at this stage. As vitellogenesis

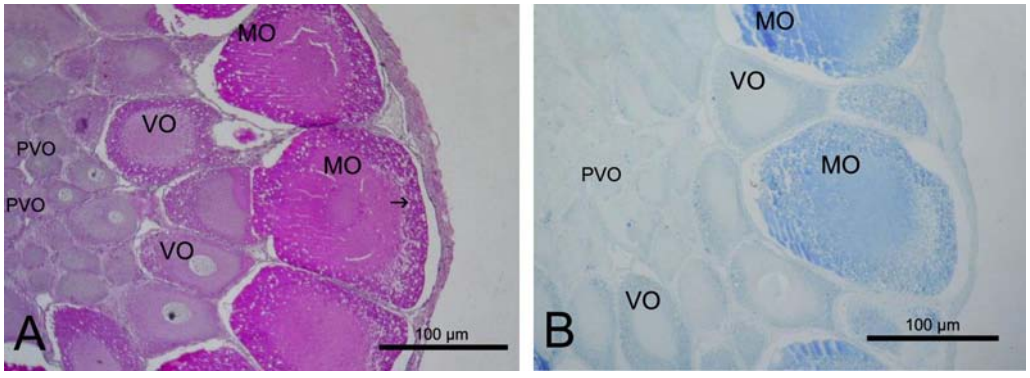

Figure 5. Light micrograph showing a transverse section through the ovary at vitellogenic stages of oocytes in *Homarus gammarus*. A. The previtellogenic oocytes (PVO) contain a few PAS positive droplets in their cytoplasm. Different from the previtellogenic oocytes, the vitellogenic oocytes (VO) contain PAS positive material throughout the cytoplasm except for the perinuclear zone. Mature oocytes (MO) contain PAS positive yolk granules (arrow) and PAS negative lipid droplets in their cytoplasm. B. The previtellogenic oocytes (PVO) stain weakly, the vitellogenic oocytes (VO) stain moderately and the mature oocytes (MO) stain stronger with Bromophenol blue. Mature oocytes (MO) contain yolk granules which stain with Bromophenol blue, and lipid droplets which do not stain with Bromophenol blue.

proceeds, the cytoplasm of oocytes becomes more heterogenic.

The mean size of the mature oocyte is 350-520 µm in diameter. Compared to other stages, larger and more yolk granules and lipid droplets can be observed in the ooplasm of the mature oocytes. The yolk granules are situated peripherally, closer to the oocyte membrane, while the lipid droplets are spread throughout the entire cytoplasm. As a result of this, the cytoplasm of the mature oocytes is much more heterogenic than the cytoplasm of the oocytes of the earlier stages. Dense yolk granules in the cytoplasm of mature oocytes are PAS positive and stain strongly with Bromophenol blue (Figure 5A,B).

The male reproductive system of *H. gammarus* consists of a pair of testes and vasa deferentia. The testes of *H. gammarus* are H-shaped and located dorsally on the hepatopancreas from the cephalothorax posteriorly to the fourth abdominal segment (Figure 6). The H-shaped testis consists of a pair of tubular, parallel lobes with a connecting bridge. The length of the anterior portion of the lobes is less than the posterior portion when measured from the connecting bridge. The average total length of the testis is 12 cm (mean carapace length 10.5 cm and mean body length 25.8 cm) and its color is often off white (Figure 6). The vasa deferentia are connected laterally to the middle of the testis lobes. The length of the vas deferens in male individuals (with average carapace length 12 cm and total body length of 25.6 cm) ranges from 32 mm to 37 mm (Figure 6).

The testis is surrounded by a single layer of thin epithelium as an outermost layer and a layer of muscle fiber, connective tissue and blood vessels underneath. The testis is basically comprised of acini

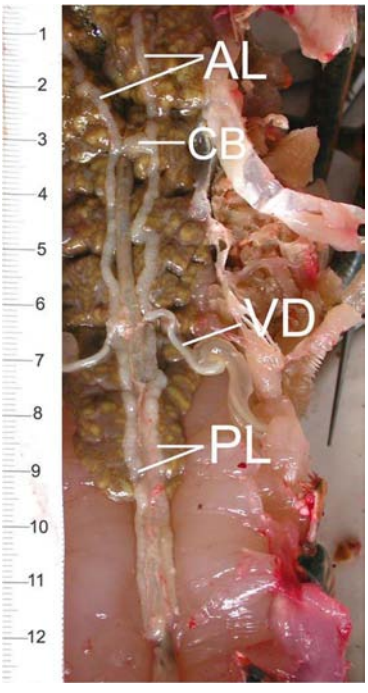

Figure 6. General view of male reproductive system in *Homarus gammarus*. Two parallel lobed testes are composed of short anterior lobules (AL) and longer posterior lobules (PL) differentiated at the central bridge. At the middle of each testis lobe, the vas deferens (VD) is connected laterally.

and associated collecting ducts. In *H. gammarus,* the clusters of acini in different regions of the testis tend to be at similar stages in the spermatogenic cycle (Figure 7A). Essentially, acini consist of generative cells and accessory cells that arise from the wall of the acini. Generally, the flattened nuclei of accessory cells stain darker with hematoxylin than the nuclei of the other

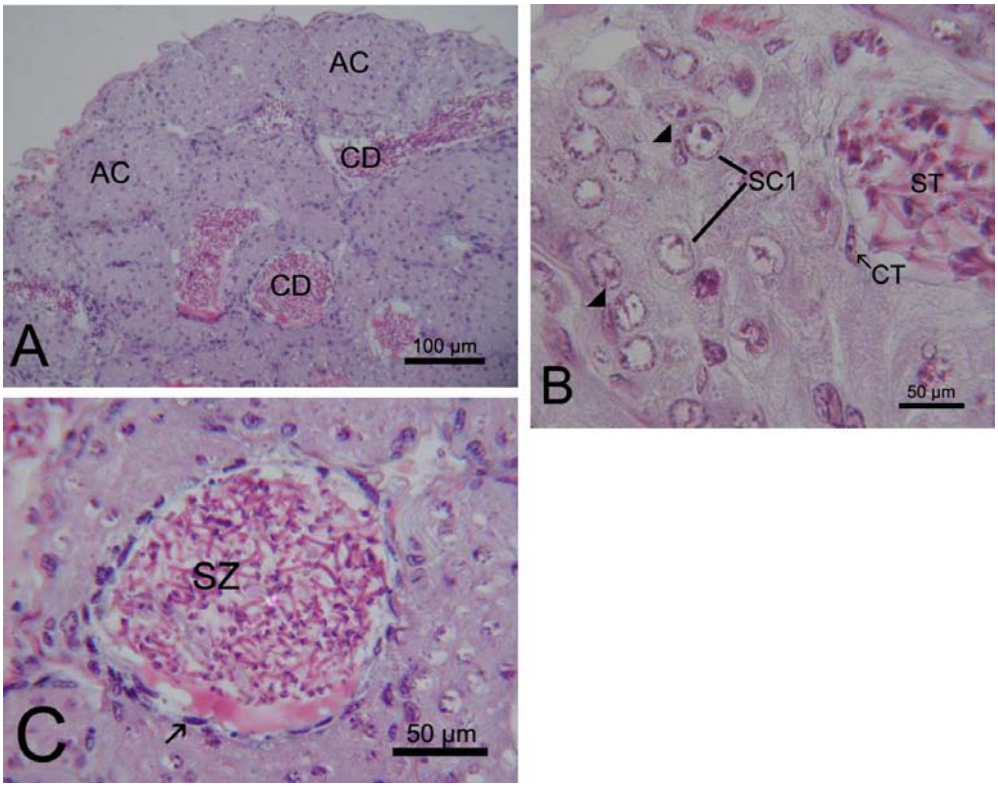

Figure 7. Cross sections of the testis in *Homarus gammarus*. A. General view of the testis showing the acini (AC) and collecting duct (CD). B. The acini showing primary spermatocytes (SC1) in meiotic synchrony, accessory cells with flattened nuclei (arrowhead) and the collecting tubule (CT) with spermatids (ST). C. Collecting ducts arising from the testis bring the spermatozoa to the vas deferens. The epithelium of the collecting duct is composed of short cubic epithelium (arrow). Spermatozoa (SZ) are embedded in PAS positive material in the collecting duct lumen.

cells in an acinus. When spermatogenesis advances in the acinus, spermatogonia first transform into primary spermatocytes, then into secondary spermatocytes and at last into spermatids, thus increasing the volume of the acinus (Figure 7B). Finally, spermatids develop into spermatozoa and accumulate at the center of the collecting tubule in each syncytial cluster (Figure 7C).

The collecting tubule is comprised of squamous epithelial cells which lay above the basal membrane, and their cytoplasm shows acidophilic characteristics (Figure 7B,C). The collecting tubules combine to form a larger collecting duct. The collecting duct with low cuboidal epithelium secretes PAS-positive material in which spermatozoa are embedded (Figure 7C). The nucleus of epithelial cells in the collecting duct is ellipsoidal and strongly basophilic. The spermatozoa that are accumulated in the collecting ducts of the testis are transferred to the ejaculatory duct *via* vasa deferentia and then ejaculated within spermatophores.

The epithelium of the vasa deferentia is surrounded by connective tissue and has the ability to secrete in some parts of the vasa deferentia. Each vas deferens can be divided into three parts according to morphological structure and secretion type: proximal vas deferens (PVD), medial vas deferens (MVD) and distal vas deferens (DVD). PVD is the first part of the vas deferens (closest to the testis) and its diameter can differ between 0.5 and 0.6 mm. PVD is surrounded by a single layer of epithelium consisting of columnar cells and there is a single layer of fibrous connective tissue beneath it (Figure 8A). The apical cytoplasm of epithelial cells in the PVD is stained with eosin but not with Alcian Blue (Figure 8A). In the PVD, spermatozoa begin to be covered by a layer (the developing spermatophore) and are embedded in a PAS-positive secretion (Figure 8B). This layer stains weakly with Bromophenol blue (Figure 8C). The second part of the vas deferens is the MVD which has a diameter ranging between 0.8 and 1.18 mm. The epithelium of MVD is highly convoluted and is composed of a single type of columnar cell with basal lobate nuclei. The epithelium in MVD is thicker than the epithelium in PVD (Figure 8A,D). The primary layer of the developing spermatophore becomes thicker, surrounds the mass of spermatozoa in the lumen of MVD

and gives a strong PAS positive reaction (Figure 8E). This layer is also moderately stained with Bromophenol blue, but not with Alcian blue (Figure 8F). The DVD is the outermost region of the vas deferens and its diameter ranges between 0.7-0.95 mm. The epithelium of the DVD is folded, as in the MVD, is highly columnar and their nuclei are located at the center of the cell (Figure 9A). The secondary layers of the spermatophore in the DVD are stained weakly with eosin and Bromophenol blue and do not give a PAS positive reaction (Figure 9A,B).

Ejaculatory duct ranks after DVD. The epithelial layer in the ejaculatory duct (ED) is highly convoluted and has ciliated columnar cells. This layer is surrounded by a thick muscular layer which contains smooth and striated muscle and is approximately 1.3 mm thick (Figure 9C). The striated muscle layer is thinner than the smooth muscle layer and is located peripherally (Figure 9D).

The spermatophore is nonpedunculate, tubular (in an uninterrupted column) and consists of a sperm mass covered with the primary and secondary layers containing acellular materials. However, acellular materials in the primary layer are different from those the secondary layer contains. The primary layer stains with Bromophenol blue moderately and gives a PAS positive reaction, while the secondary layer stains with Bromophenol blue weakly and does not give a PAS positive reaction. Both layers are thicker in the DVD than in the MVD.

4. Discussion

The studies previously published on *Homarus gammarus* concentrate on development, dispersion, the reproductive cycle, and egg production. The female reproductive system of *H. gammarus* has a pair of ovaries, a pair of oviducts and a receptacle in order to store sperm (seminal receptacle), as previously seen in

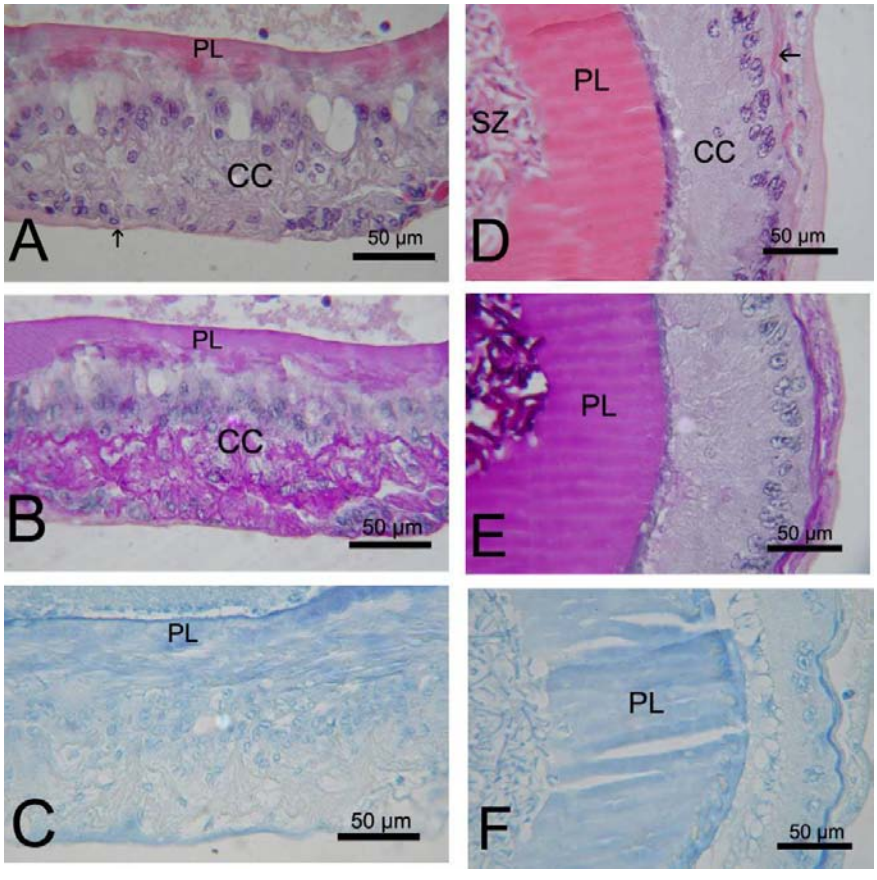

Figure 8. Light micrograph transverse section through the vas deferens of *Homarus gammarus.* A. Proximal vas deferens (PVD) consisting of fibrous connective tissue (arrowhead) and columnar cells (CC) whose apical cytoplasm stains with eosin and forms the primary layer of the spermatophore (PL). B. Columnar cells of the PVD secrete a PAS positive material which forms the primary layer of the spermatophore (PL). C. This primary layer (PL) also stains with Bromophenol blue. D. Medial vas deferens (MVD) consisting of highly columnar cells (CC) with basal nuclei and surrounded by fibrous connective tissue (arrowhead) and contained spermatozoa (SZ) mixed with a dense acellular material. The primary layer of the spermatophore (PL) is thicker than in the PVD. E. Primary layer of spermatophore (PL) gives a strongly PAS positive reaction. F. Primary layer of spermatophore (PL) stains with Bromophenol blue.

H. americanus [14]. The ovary of *H. gammarus* extends from the anterior end of the cephalothorax to the fifth abdominal somite as in *H. americanus,* [15], and when ovaries are fully mature they can extend to the eyestalks. The eggs are released from the genital pore on the coxa of the third walking legs.

4.1 Ovary

The ovary of the lobster *H. gammarus* is H-shaped, as in *H. americanus* (Homaridae), *Nephrops norvegicus* (Nephropidae) and *Cherax quadricarinatus* (Parastacidae), whereas *Astacus leptodactylus* (Astacidae) and *Procambarus clarkii* (Cambaridae) have Y-shaped ovaries [15-19].

The ovary is surrounded by a wall consisting of thin squamous epithelium, connective tissue, muscle and blood vessels. It has been suggested that contraction of the ovary wall is needed to expel the mature eggs [14]. The ovary wall of *H. gammarus* is not as thick as *H. americanus* and is as thin as that in *C. quadricarinatus* and *P. clarkii*, and it is formed by prolonged straight muscle fibers. *A. leptodactylus* does not contain any muscle in the ovary wall [20-23]. The oviduct epithelium in *H. gammarus* produces a secretion which gives a PAS positive reaction from its apical surface to the lumen but it does not stain with Bromophenol blue. Ando and Makioka [21] state that the epithelium of the oviduct may be related to fertilization that may occur in the oviduct. However, it is strongly suggested that the oviduct epithelial secretion rather plays the role of helping in the destruction of spermatophores and the protection of eggs against microorganisms [24], since only external fertilization is observed in *H. gammarus*, *C. quadricarinatus* [25] and *A. leptodactylus* [23].

The location of the region where the germinal epithelium exists in the ovary varies among different species of decapods. In crustaceans, five types of germarium regions are defined: 1) throughout the

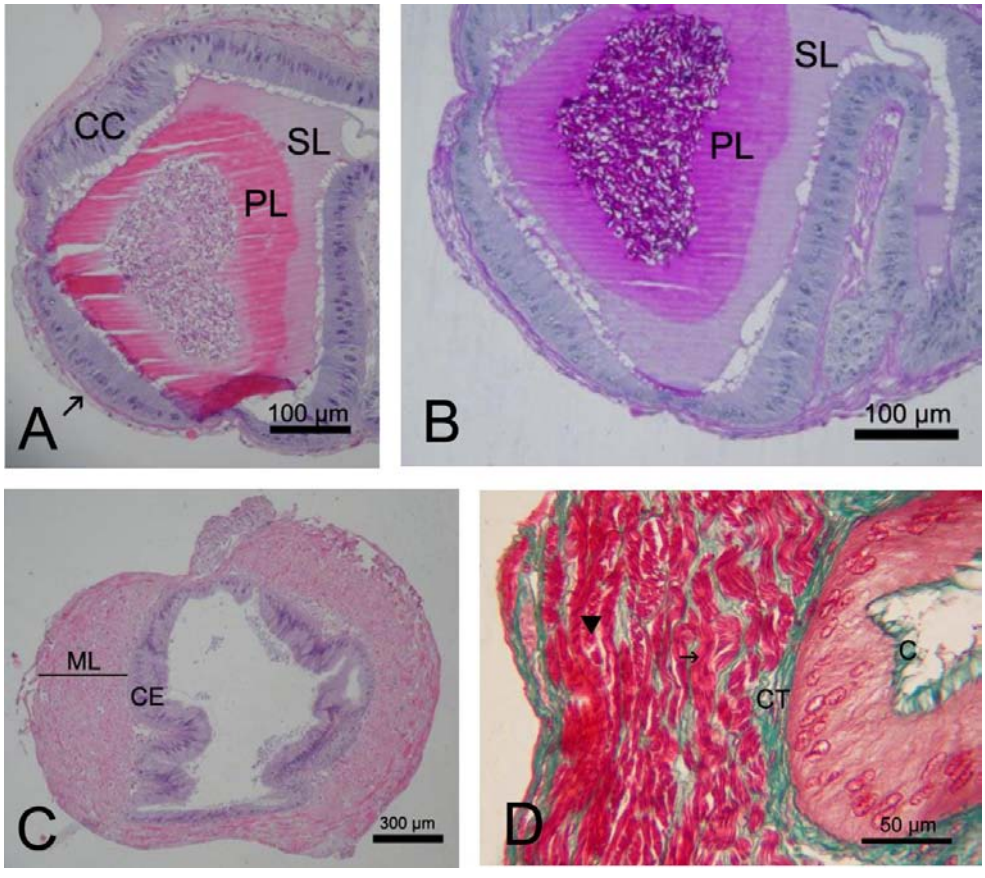

Figure 9. Light micrograph transverse section through distal vas deferens and ejaculatory duct of *Homarus gammarus*. A. Distal vas deferens (DVD) consisting of highly columnar cells (CC) with central nuclei and surrounded by fibrous connective tissue (arrowhead). Secondary layer of spermatophore (SL) becoming obvious and stains weakly with eosin. B. Primary layer (PL) gives a PAS positive reaction, while secondary layer (SL) does not give a PAS positive reaction. C. The ejaculatory duct is composed of highly convoluted epithelium (CE) that surrounds the lumen (L). There is a thick muscular layer (ML) under the epithelium. D. The epithelial cells have cilia (C) on the side of the lumen (L) and connective tissue (CT) on the basal side. The thick muscle layer which is located under the connective tissue is composed of striated muscle (arrowhead) and smooth muscle (arrow).

ovary periphery, 2) in the shape of a thin band in the lateral periphery or ventral periphery, 3) in the form of a germinal band in the center, 4) in the form of germ clusters and scattered throughout the ovary, and 5) in the form of clusters only in the ovary periphery [16]. The germarium, as a germinal cord, extends from anterior to posterior at the center of the ovary in *H. gammarus;* as seen in *H. americanus* [26], *N. norvegicus* [27], and *P. clarkii* [21]. The acini develop in the germarium radially toward the periphery in *H. gammarus*. While the oogonia are present in the germarium, the previtellogenic, vitellogenic and mature oocytes are observed more towards the periphery.

Only the follicular cells are nongerminative accessory somatic cells in the decapod ovary, and they are dispersed sporadically during development of the germarium. While in the germarium, the follicular cells are generally round and ellipsoid but become flat when they attach to the oocyte. During vitellogenesis, follicular cells can function in different ways in yolk production [16]. While the elliptical follicular cells, scattered in the germarium, can be present sporadically around the previtellogenic oocytes in *H. gammarus*, these cells surround the entire vitellogenic oocyte as a layer in *H. americanus* [28], *C. quadricarinatus* [25] and *A. leptodactylus* [23].

The oogonia in *P. clarkii* [21,29], *A. leptodactylus* [23] and *C. quadricarinatus* [30] contain thread-like and granular basophilic chromatin material in their very large nuclei.This is very similar to that seen in *H.gammarus.* In addition, at proliferation stage in *H. gammarus* the differentiation of the chromatin material from a filamentous pattern towards a granular structure and the weak eosinophilic nature of the cytoplasm is similar to *C. quadricarinatus* [30]. The fact that the oogonia and follicle cells at this stage produce a PAS negative reaction and do not stain with Bromphenol blue indicates that they do not contain carbohydrate and protein yet.

In *H. gammarus*, previtellogenic oocytes have amorphous basophilic cytoplasm whereas vitellogenic oocytes have eosinophilic cytoplasm. In addition, the fact that the previtellogenic oocytes of *A. leptodactylus* at this stage produce a PAS positive reaction but do not stain with Bromophenol blue, indicates that these cells begin to store carbohydrate before protein and lipid; as recorded in *C. quadricarinatus* [30]. Vitellogenic oocytes of *H. gammarus* are smaller than those of *A. leptodactylus* [23], *P. clarkii* [21] and *C. quadricarinatus* [25,30]. The yolk material that accumulates in the cytoplasm of the vitellogenic oocytes in *H. gammarus* during vitellogenesis stains with Bromophenol blue and produces a PAS positive reaction. This shows that the yolk contains protein and carbohydrate. The droplets that are seen in *H. gammarus* for the first time in the vitellogenic oocytes, do not stain with Bromophenol blue and Alcian blue and produce a PAS negative reaction. This is indicative of lipid-like characteristics, as seen in *A. leptodactylus* [23,31-33]. Kulkarni *et al*. [34] do not mention lipid droplets in their definition of vitellogenesis in *P. clarkii.* However, Ando and Makioka [21] stated that lipid droplets were present in the cytoplasm of the largest previtellogenic oocytes in *P. clarkii.* As a result, lipid is mostly observed first in the vitellogenic stage, which is consistent with the results of the present study. The mature oocytes store yolk intensely and are ready for spawning in *H. gammarus,* as in other taxa. At this stage, the mature oocytes of *H. gammarus* have a smaller size compared with those of *Virilastacus araucanius* [35], *Orconectes limosus* [33], *C. quadricarinatus* [25,30,36], *P. clarkii* [21] and *A. leptodactylus* [23]. The yolk granules are abundant in the periphery of the mature oocytes, closest to the cell membrane. It is observed that in *H. gammarus* some yolk granules in the cytoplasm of mature oocytes give a PAS positive reaction and stain more strongly with Bromophenol blue compared to the earlier stages in *C. quadricarinatus* [30], *P. clarkii* [21] and *A. leptodactylus* [23].

4.2 Testis

A H-shaped testis is observed in the Nephropidae (Superfamily Nephropoidea). The testis morphology of *H. gammarus* shows similarities to *H. americanus* [14] and *N. norvegicus* [37], both species of Nephropidae, but *C. quadricarinatus* [22] (Parastacidae) differs from *A. leptodactylus* [24] (Astacidae).

The posterior testis lobules are longer than the anterior testis lobules and a bridge connects the two parallel lobes in the testis of *H. gammarus;* as in *H. americanus* [14].The vas deferens connects to the testis at the center in *H. gammarus* and the vas deferens is not longer than the individual, differing from Astacidae and Cambaridae. The entire male reproductive system, from the testis to the ejaculatory duct, is surrounded by squamous epithelium at the outermost layer.

In decapods, the stages of spermatogenesis are either synchronized or asynchronized depending on the species. Spermatogenesis in *H. gammarus* is synchronous like in *A. leptodactylus* and *P. clarkii* [21,24]. In contrast, *C. quadricarinatus, Parastacus defossus* and *Samastacus spinifrons* all show asynchronous spermatogenesis [22,38,39].

The testis of *H. gammarus* is very similar to other decapods in that it is comprised of acini and collecting ducts. Acini consist of spermatogenic cells and accessory cells; the latter differ from other cells in the acinus because they possess flattened and basophilic

nuclei. Spermatids produced in each acinus are transmited to collecting ducts and then to the proximal vas deferens (PVD). The spermatozoa that pass to the vas deferens are surrounded by spermatophoric layers. Eventually, the spermatophore passes through the PVD, MVD and DVD and is extruded from the ejaculatory duct of the male to the seminal receptacle of the female. While in *H. gammarus* the vas deferens has morphological and histological similarities to *H. americanus* and *N. norvegicus*, it has less similarity with *Cherax albidus*. Depending on the species, the vas deferens may be divided into 3 to 10 different regions [40]. In *H. gammarus* the vas deferens is divided into 3 regions (proximal, medial, distal vas deferens), as in most of the individuals in the infraorder Astacidea. The number of vas deferens regions in *H. americanus* is five, consisting of two proximal regions, one medial region and two distal regions [14].

The proximal vas deferens shows continuity with the collecting ducts of the testis [41]. As in Nephropoidea, the proximal vas deferens in *H. gammarus* is composed of basophilic, multi-nucleated, cylindrical epithelium [19]. In the PVD the connective tissue and muscle fibers surround the epithelial layer in *H. gammarus*, while the same region consists of very thick connective tissue and striated muscles in *H. americanus* [14]. In *H. gammarus* the primary spermatophore layer begins to cover the sperm mass in the PVD as seen in *C. quadricarinatus* [22], however this layer only appears in the MVD in *A. leptodactylus* [24]. The primary spermatophore layer begins to surround the sperm mass in the second segment of the PVD in *Homarus americanus* [14].

In *H. gammarus* the MVD is wider than the PVD; as seen in *H. americanus* [14]. The epithelial cells of the MVD of *H. gammarus* [24] are higher than the epithelial cells of the PVD. Besides this, the epithelial cells of the MVD vary from flat to cubic in *C. quadricarinatus* [22].

Talbot and Helluy [14] reported that the DVD is also called an ejaculatory duct, but in *H. gammarus,* the DVD and ejaculatory duct are structurally rather different from each other. While the epithelium of the DVD consists of short columnar cells in *H. americanus* [14], it consists of higher columnar cells in *H. gammarus*. The formation of the second layer of the spermatophore and completion of the spermatophore itself occurrs in the beginning of the DVD in *H. americanus*, the second layer of the spermatophore continues to form along with the DVD in *H. gammarus*.

The ejaculatory duct is composed of a thick striated muscle layer in *H. americanus* [14], while this layer is composed of both smooth muscle and striated muscle in *H. gammarus*. Also the ejaculatory duct is covered with a ciliated epithelium in *H. gammarus,* however, the epithelium of the ejaculatory duct in *H. americanus*, *C. quadricarinatus,* and *A. leptodactylus* is not ciliated [14,22,24].

Storage of the spermatophore, after transfer from the male, differs among the different groups of decapods. While the spermatophore is stored in the genital duct of the female in the groups in which internal fertilization is observed, it is stored in the ventral thoracic cavity, or somewhere in the path of unfertilized eggs, in the groups in which external fertilization is observed (*e.g. H. gammarus*) [17,42,43]. The spermatophore has a simple structure in the groups with internal fertilization, while the spermatophore has a more complex structure when it is stored outside of the body in groups where external fertilization is observed [44,45]. There are three types of spermatophores in decapods: the first is pedunculate and it is present in nearly all Anomura, except a few species in Hippidae [46,47]; the second is tubular and is found in Astacidea such as *H. gammarus, H. americanus, A. leptodactylus, P. leniusculus* and *Enoplometopus occidentalis* [42,48,49]; and the third is the simplest shape, being small and ellipsoid or spherical, and is found in brachyuran crabs [17,50].

The number of spermatophore layers in the Astacidea may also vary between species. While there are three spermatophore layers in *P. leniusculus* [51], there are two spermatophore layers in *H. gammarus A. leptodactylus, C. quadricarinatus,* and *H. americanus* [14,22,24]. The sperm mass in the lumen of the spermatophore in *H. gammarus* gives a strong PAS positive reaction which is the same as in *A. leptodactylus, C. quadricarinatus* and *C. albidus* [22,24]. The primary layer of the spermatophore gives a PAS positive reaction in *H. gammarus,* as in *P. leniusculus, Cherax cainii, C. albidus,* and *C. quadricarinatus* [51-53] and also is moderately stained with Bromophenol blue, showing that this layer is glycoproteinaceous in nature [13,54]. The secondary layer of the spermatophore gives a PAS positive reaction in *C. quadricarinatus,* but it gives a PAS negative reaction in *H. gammarus* and *A. leptodactylus* [24]. This layer also stains lightly with eosin and Bromophenol blue, indicating an acidophilic and proteinaceous structure in *H. gammarus*, but it stains with alcian blue in *A. leptodactylus*, showing that it has a hyaluronic structure.

New microanatomical and histochemical information on the ovary and testis structure of this commercially high-priced lobster species, *H. gammarus,* has been revealed with this study. This information of the female and male reproductive system helps to better understand the management and rearing in culture of this species and gives opportunity to compare the phylogenetic relations of *H. gammarus* by casting light on future studies.

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