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THE GONOPOD TEGUMENTAL GLANDS OF SNOW CRAB (*CHIONOECETES OPILIO*) ARE ACCESSORY REPRODUCTIVE GLANDS

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ABSTRACT To date, the role of the tegumental glands found in the first gonopod of brachyuran crabs has been a matter of conjecture. In order to more clearly understand the nature and ultimate function of these glands, histological and histochemical studies were performed on 17 male snow crabs, *Chionoecetes opilio* (O. Fabricius), captured in the Baie des Chaleurs, New Brunswick, Canada. Mature (M) and immature (IM) individuals were differentiated based on the carapace width (CW): cheliped height (CH) ratio. To assess the developmental trajectory of the glands, the immature crabs were subdivided into 3 groups, small immature (<40 mm CW), medium immature (40–70 mm CW), and large immature 70–100 mm CW). The precise distribution of the glands within the first gonopod was determined via serial sections (7–10 μm). The following histochemical tests were performed on the subsequently revealed glandular region of the first gonopod: Sudan black and Nile blue for lipids, orange G for aminated substances, alcian blue for acid mucopolysaccharides, and periodic acid-Schiff for neutral mucopolysaccharides (MPS). The volume fraction of the gonopod glandular region occupied by glands was assessed using stereologic counts.

The glands were determined to be of the rosette type, and restricted to a specific region at the base of the endopodite. Ducts leading from these glands to the cuticle of the ejaculatory canal only were clearly visible in medium immature to mature individuals; these ducts connected to pores in the cuticle. Cuticular pores and ducts were not observed in small immature crabs. The volume fraction of the glands increased in each successive maturity category, with a mean of 0.8% in small immature crabs and a mean of 8% in mature crabs. The glands contained either acid or neutral mucopolysaccharides, or a mixture of both. The pores of the ejaculatory canal contained similar secretions. These observations support the conclusion that the first gonopod tegumental glands in *C. opilio* are accessory sex glands.

KEY WORDS: *Chionoecetes*, reproduction, gonopod, tegumental gland

INTRODUCTION

Majid crabs of the genus *Chionoecetes* are a major fishery species of the Northern hemisphere (Anonymous 1989); in Atlantic Canada, the snow crab (*Chionoecetes opilio*) fishery accounted for catches of 18,580 MT in 1993, with a commercial value of nearly $140 million (Department of Fisheries and Oceans 1995). As a relatively recent fishery (begun in the 1970s), it was eventually recognized that a firm understanding of reproductive processes was necessary for the enlightened management of snow crab stocks (Bailey and Elner 1989). Anatomical, histological, behavioral, and physiological studies have progressively elucidated the complex reproductive biology of this species, which is characterized by several potential pathways (for review, see Elner and Beninger, in press).

As in all decapods, fertilization in snow crab is accomplished via the male's first and second pleopods, which are variously modified to form gonopods (Bauer 1986). Despite their obviously crucial role in reproduction, as well as their significance in taxonomic determination and phylogeny (Hartnoll 1975, Chambers et al. 1980, Bauer 1986, Martin and Abele 1986), brachyuran gonopods have been the object of surprisingly little study (see Beninger et al. 1991 for review and references). The functional anatomy and histology of the mature snow crab gonopods were documented by Beninger et al. (1991). Among other findings, the presence of a rosette-type glandular system was reported within the first gonopod. This system was present in the first gonopod only, and was shown to be connected to pores in the ejaculatory canal cuticle; other cuticular regions were not linked to the glands and contained no pores. These observations led Beninger et al. (1991) to propose that the glandular system was related to reproductive function, and being outside the anatomical continuum of the testes-vas deferens, they could therefore be considered accessory sex glands.

The vocabulary and ascribed functions of glands associated with the crustacean exoskeleton were somewhat confusing (see Beninger 1991 for discussion of this point), until Talbot and Demers (1993) published a comprehensive review of the subject. It was thenceforth clear that the glands of the snow crab first gonopod were tegumental glands of the rosette type. Debate continued, however, concerning their functional status as accessory sex glands, and in a recent review, Subramoniam (1993) concluded that even more data would be required before assigning such a status to these glands. In essence, it would be necessary to determine whether the glands are associated with cuticle hardening and maintenance, or whether they are associated specifically with reproduction.

The purpose of the present study was thus to further investigate the function of the tegumental glands of the male first gonopod in snow crab, *C. opilio*. The approach was twofold: more detailed histological and histochemical work to better document the gland-ejaculatory canal relationship and the nature of the gland secretions, and a developmental study to determine whether the degree of gland development is related to carapace hardening and consolidation (moult frequency inversely proportional to age) or to sexual maturity.

MATERIALS AND METHODS

Specimens and Sampling

A total of 17 male snow crabs were recovered from traps in the Baie des Chaleurs (New Brunswick, Canada), between November 1993 and November 1994, at depths of 60–120 m. Six crabs obtained in November 1993 were maintained until dissection in July 1994 in open-circuit holding tanks, supplied with water from the Baie des Chaleurs (mean temperature = 1°C, mean salinity = 29‰). The tanks were covered in order to simulate the extremely low light intensity of the crabs' natural habitat. They were fed *ad libidum* with shrimp or smelt once weekly, and detritus was removed the following day. Seven crabs were obtained in June 1994 and dissected the same week, while four others were obtained in November 1994, of which two were dissected the next day. The two remaining crabs were maintained as above and dissected in December 1994 (for lipid tests).

Biometric Measurements and Maturity Categories

The maximum carapace width (CW) and cheliped height (CH) of all crabs were measured to the nearest millimeter using Vernier calipers. The CW:CH ratio was calculated and compared to a previously established scale in order to distinguish between morphometrically mature and immature crabs (Conan and Comeau 1986).

In order to quantify gland system development, the morphometrically immature crabs were further subdivided into 3 size groups, based on carapace width: small immature (SI: <40 mm), medium immature (MI: 40–70 mm), and large immature (LI: 70–100 mm). In order to compare homogeneous groups rather than a continuum of sizes, variation in specimen size within each group was minimized The actual means and ranges were: SI 27.3–30.6, $\overline{X}$ = 28.8; MI 53.6–66.0, $\overline{X}$ = 61.1; LI 88.2–99.5, $\overline{X}$ = 95.4.

Dissection, Fixation, Histological Processing

The first gonopods of each crab were removed at the base of the protopodite and fixed as described below. The vas deferens was also removed and fixed, in order to effect whichever histological tests were found positive in the gonopod glands. The purpose of this procedure was to attempt to establish whether any secretions of the glands which might be transferred to the spermatheca during copulation could be confused with secretions from the vas deferens.

Prior experience had shown the first gonopods to be particularly problematic for micotome sectioning, due to the considerable difference in hardness between the cuticle and the very loose internal tissue. In an attempt to alleviate this problem, 3 fixation solutions were tested: Helly's, Böhm Strenger, and aqueous Bouin's (Vacca 1985). Of these, only the aqueous Bouin's allowed sectioning. Penetration of the fixative was enhanced by first bisecting the gonopod at the distal extremity of the pinnate setae distribution, and then piercing the cuticle on the face opposite to the ejaculatory canal with entomological needles. The fixation was then performed under vacuum, in order to eliminate air from the ejaculatory canal which had been introduced during dissection. Despite these precautions, the internal tissue was often separated from its points of attachment to the cuticle during sectioning. The best sections were obtained from gonopods prepared as above and left for 3 months in aqueous Bouin's fixative. No improvement was obtained from resin-embedded gonopods, which presented incomplete matrix penetration.

One gonopod each from a mature and large immature crab were fixed in calcium-formol (Vacca 1985) for lipid histochemistry.

All gonopods except those tested for lipids were dehydrated in an ascending ethanol-xylene series and embedded in paraffin. Serial sections were performed on the first 10 embedded gonopods, in order to determine the exact distribution of the glands. The 5 remaining paraffin-embedded gonopods were serially sectioned at 10 μm within this region. Gonopods tested for lipids were frozen on cryotome stubs and cryosectioned at 16 μm within this region.

Histochemistry

The histochemical tests performed on the gonopod sections are indicated in Table 1. Due to the non-specific nature of the Orange G test (all aminated substances were targeted), no negative control was used in this case only. The tests were chosen to cover the major groups of biochemical constituents likely to be either secreted or stored. Staining times were: alcian blue, 30 min; Schiff, 1 sec (Fisher Scientific) or 10 min (BDH); Sudan black, 10 min; Nile blue 15 min. Alcian blue was contrasted either with trioxyhematein (Gabe 1968) or nuclear fast red (Vacca 1985). A combined alcian blue/periodic acid-Schiff staining procedure was performed in order to identify glands containing a mixture of acid and neutral mucopolysaccharides; staining times were as above, with alcian blue preceding Schiff reagent. Due to the extremely small size of gonopods from the small and medium immature size groups (approx. 8–9 mm long, glandular region approx. 1 mm), cryostat sectioning for lipid tests could only be performed on mature and large immature individuals.

Stereology

Stereological counts were performed on gonopod sections of 3 males from each maturity category. A 540-point matrix (each point separated by 1 cm) was superimposed on a video monitor screen output from the microscope. Counts were performed at 40× for all categories except small immatures, which were done at 100×. The volume fraction of the gonopod occupied by glands (gland volume fraction) was determined for 3 complete gonopod

TABLE 1.

***Chionoecetes opilio*. Histochemical tests performed on gonopod tegumental glands.**

Substance	Test	Positive Control	Negative Control	Reference
Lipids	Sudan black Nile blue	Hepatopancreas	Lipid extraction	High 1984
Proteins	Orange G	Muscle	–	James and Tas 1984
Acid MPS	Alcian blue	Hepatopancreas	Amylase digestion	Vacca 1985
Neutral MPS	PAS	Hepatopancreas	Amylase digestion	Vacca 1985

sections for each individual, and the individual mean was then calculated (Beninger 1987). The mean gland volume fraction and standard deviation were then calculated from the individual means of the 3 crabs of each maturity category.

RESULTS

Gland Distribution, Aspect, and Histochemical Features

Serial sections of gonopods from all size categories showed the glands to be restricted to a specific region at the base of the endopodite; this distribution is not characterized by any particular external anatomical markers (Fig. 1). The glands were of the rosette type (Talbot and Demers 1993), and were present to varying degrees in all crabs examined (Fig. 2), with the exception of the smallest immature individual. The glands were fewest in the small immature category; however, cellular aggregates visible as local concentrations of nuclei which may have been gland anlagen were observed using nuclear red counterstain in both the small and medium immature categories. Glands were separated by loose connective tissue. Glands contained either acid or neutral mucopolysaccharides, or a mixture of both types of mucopolysaccharide. The glands were negative for lipid, and slightly positive for aminated substances (consistent with the positive mucopolysaccharide results). The vas deferens contained a mixture of acid and neutral mucopolysaccharides. The neutral MPS were visible as numerous red spheres in the surrounding acid MPS, contrasting with the mixed MPS in the gonopod glands, which had a more homogeneous appearance.

With the exception of the smallest immature crab (which had no glands, as noted above) the glands were grouped around the ejaculatory canal, while the gonopod muscles were situated opposite the ejaculatory canal. In mature, large and medium immature categories, the ejaculatory canal cuticle possessed pores, which contained substances exhibiting the same histochemical properties and microscopic appearance as the glands (Fig. 2.3, 2.4). The glands were connected to the pores via a network of ducts (Fig. 2.2, 2.3, and 2.4); no pores or ducts were present in the cuticle outside of the ejaculatory canal. Distinct dilatations of the ducts were observed on the proximal face of the cuticle. In the small immature category, no pores or dilatations were observed in histological sections of the cuticle, even in crabs which presented glands.

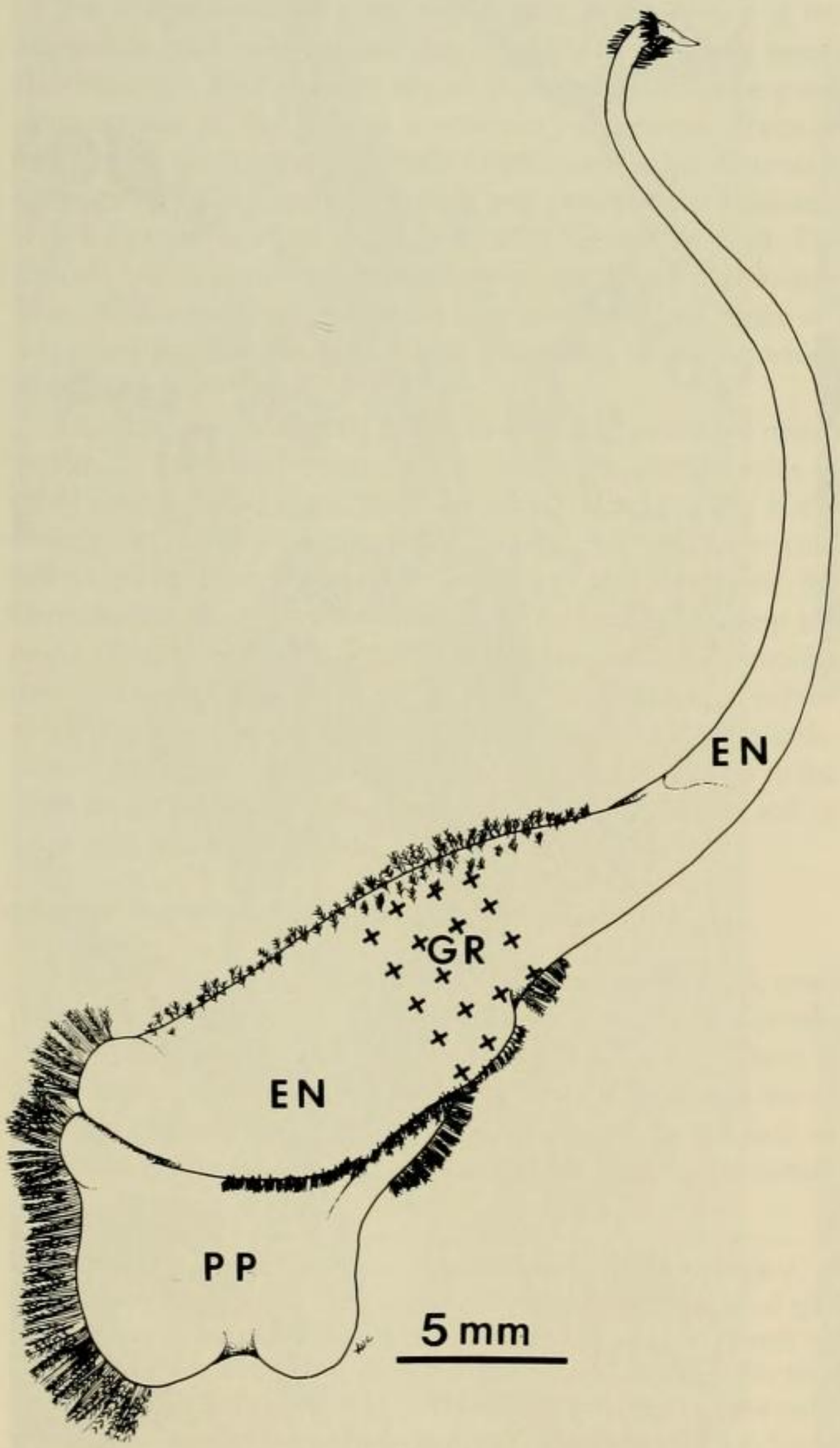


Figure 1. General morphology of mature *Chionoecetes opilio* first gonopod. EN, endopodite; GR, gland region; PP, protopodite.

Stereology

The mean gonopod gland volume fractions for each maturity category are presented in Figure 3. The lowest volume fraction was found in the small immature category; there was a fivefold increase between the small immature and medium immature categories, and the volume fraction continued to increase in the subsequent maturity categories. The gland volume fraction of the mature crabs was an order of magnitude larger than that of the small immature category (8% vs. 0.8%).

DISCUSSION

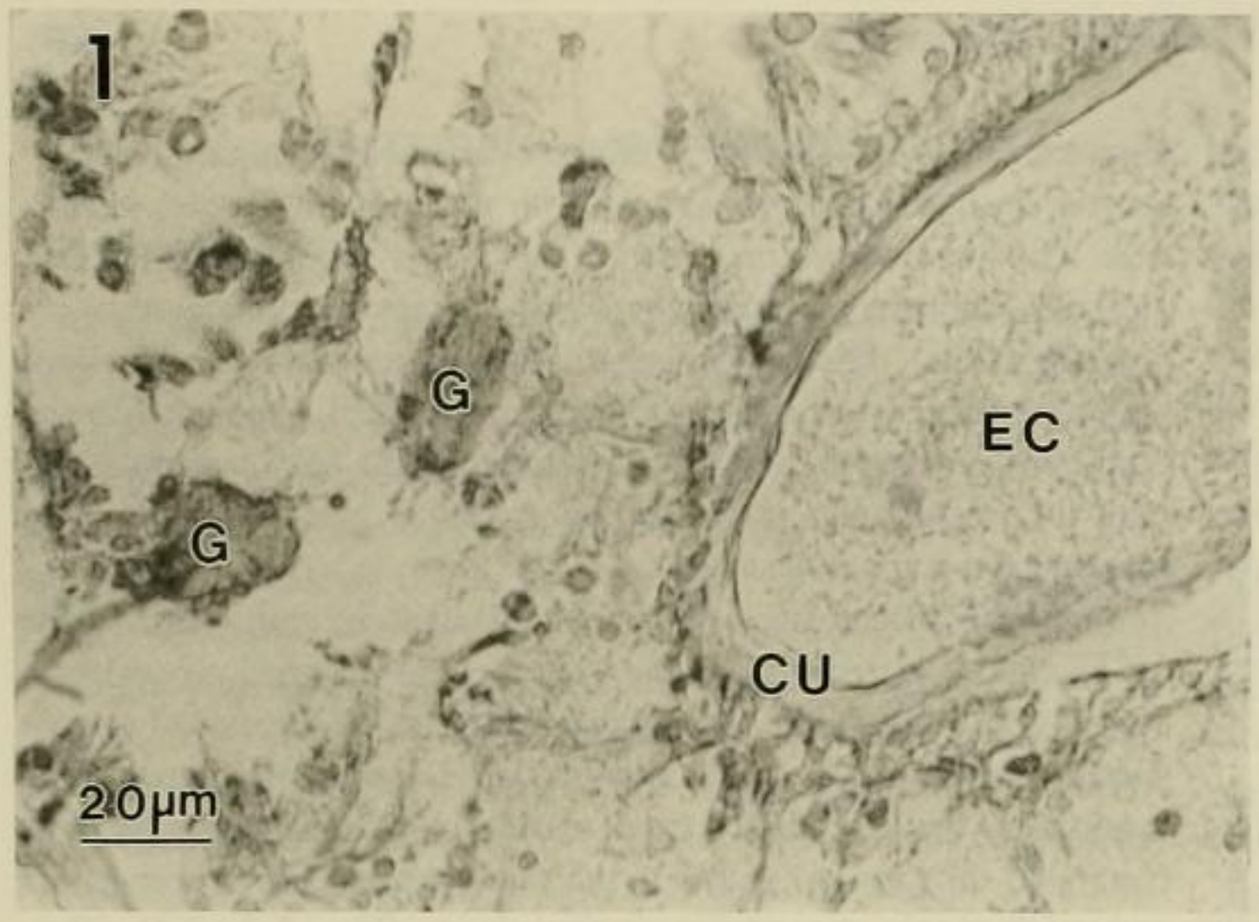
Gonopod Tegumental Glands as Accessory Reproductive Glands

Taken together, the results of the present study are congruent with a reproductive function for the gonopod tegumental glands in *C. opilio*. As was previously demonstrated in adult snow crabs (Beninger et al. 1991), the duct system and associated cuticular pores are directed exclusively toward the ejaculatory canal in all but the most immature individuals (small immature size group), which lacked pores and had very few glands. The absence of such pores in these individuals, and in the rest of the cuticle of all other specimens, is inconsistent with the competing hypothesis of cuticle maintenance or hardening. Finally, the direct relation between volume fraction of the glands and maturity category is unequivocal; it is antithecal to functions associated with cuticle hardening or maintenance, since younger animals moult more frequently than older ones and hence would require the opposite volume fraction-maturity relationship.

Ontogeny of Gonopod Gland System

Cell aggregates observed in the small and medium immature individuals may represent gland anlagen, as reported for *Callinectes sapidus* (Johnson 1980). The glands differentiate, become more numerous, and the associated duct system develops as the individual grows. Detailed ultrastructural investigation would be

1


G
G
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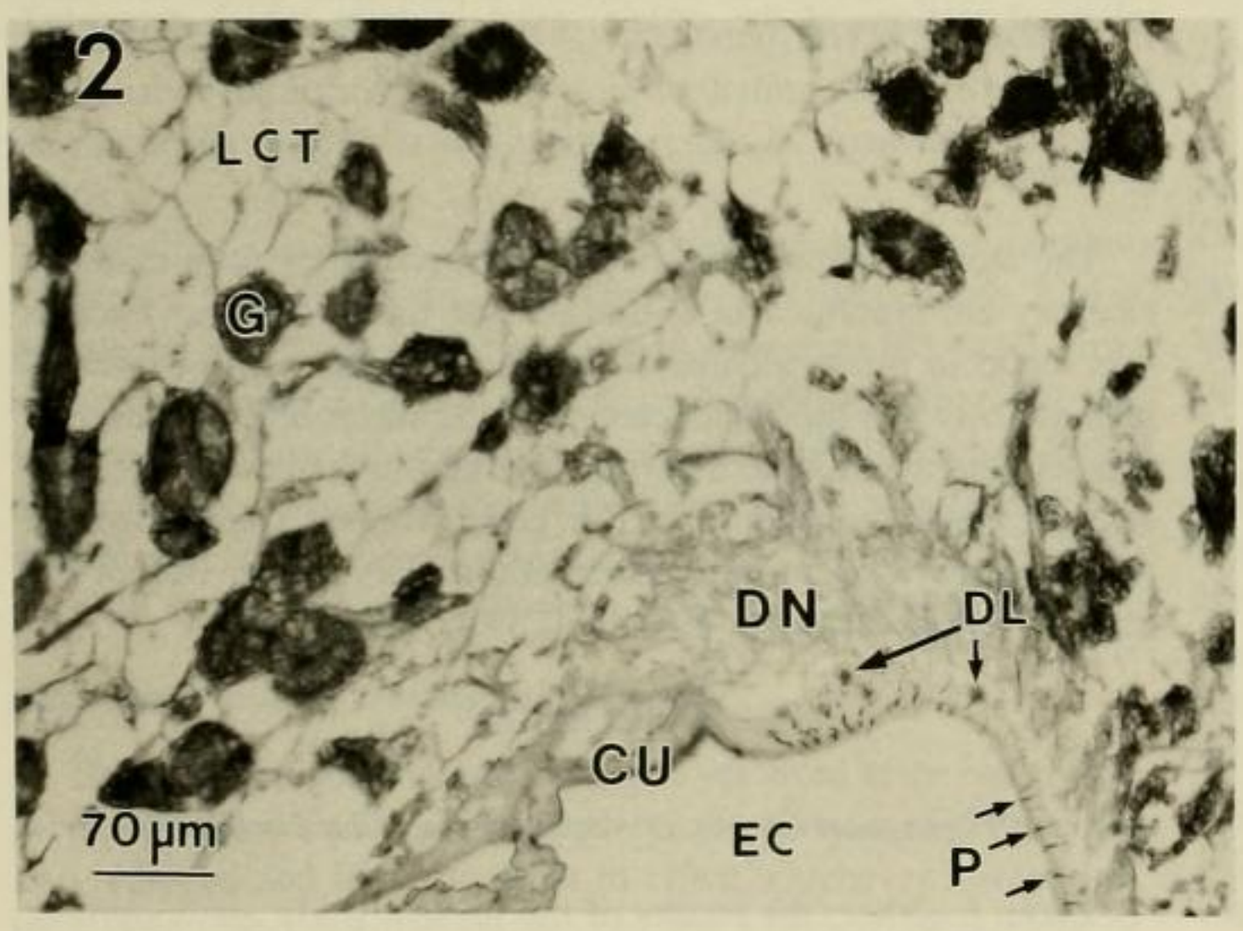
2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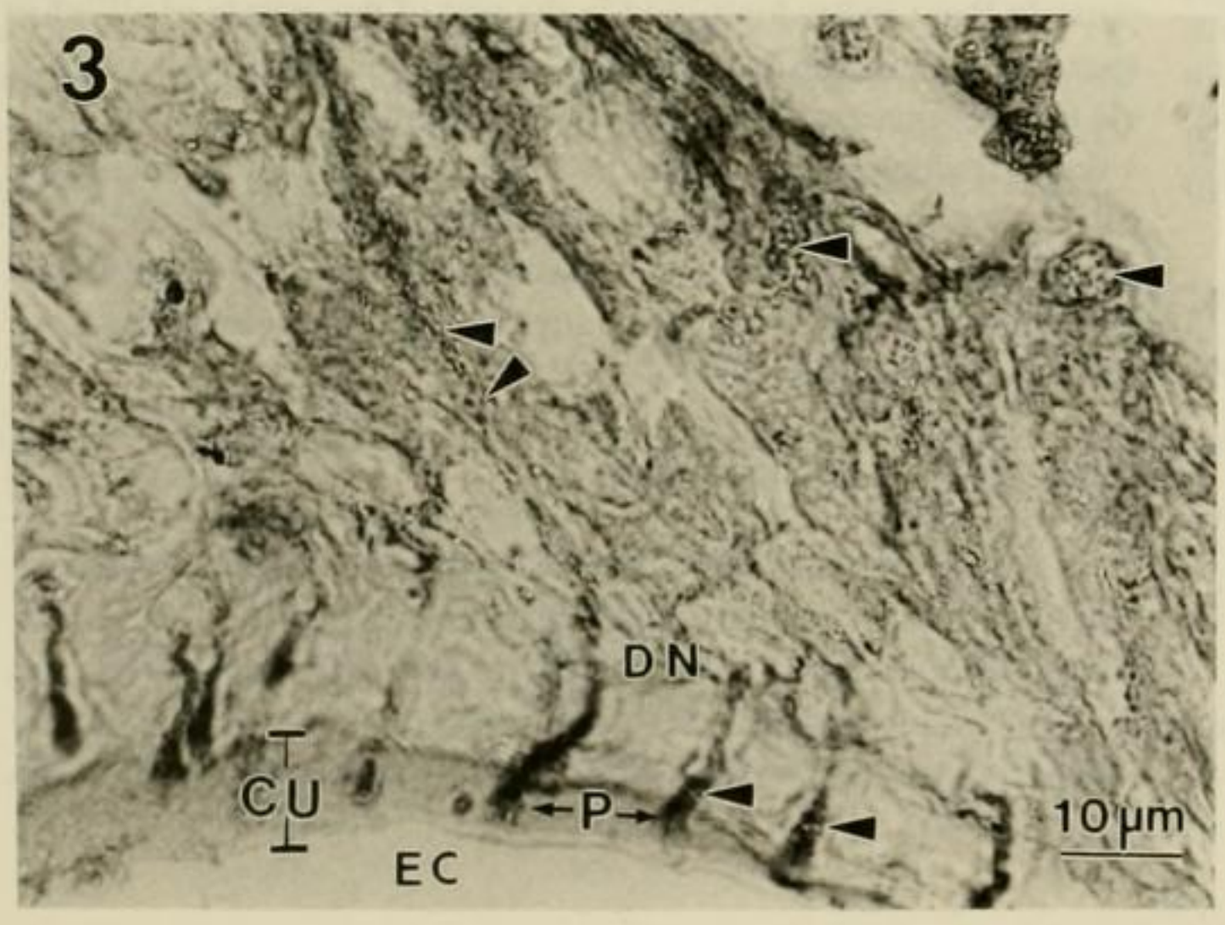
3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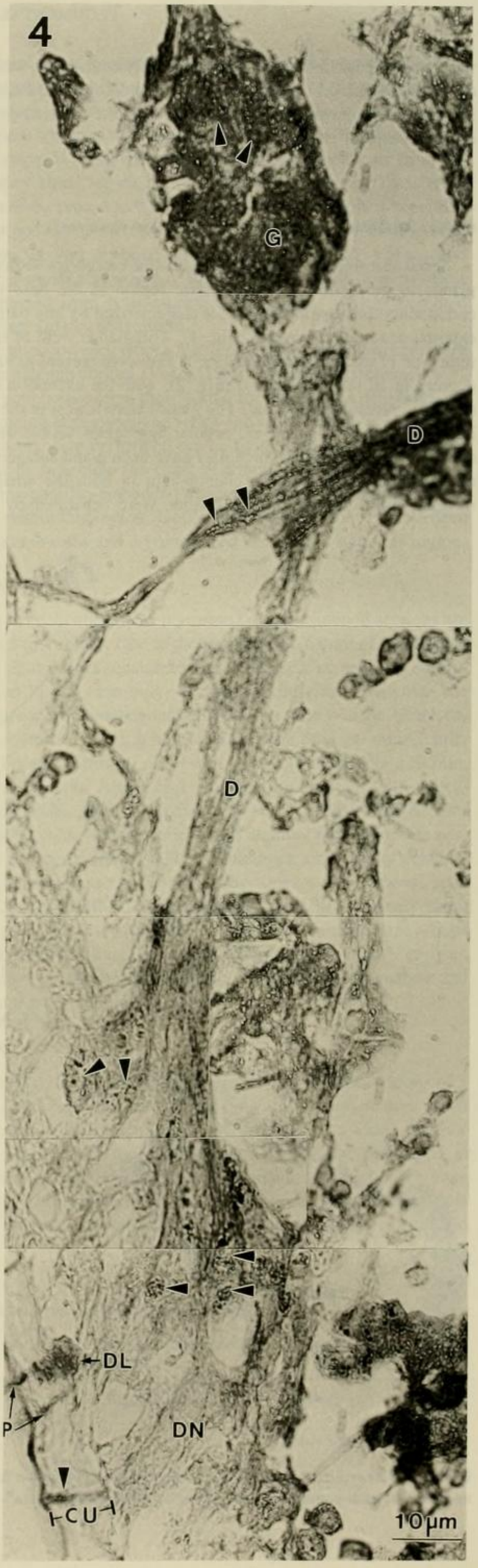
4


G
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DN
CU
10μm

TABLE 2.

***Chionoecetes opilio*. Results of histochemical tests performed on gonopod tegumental glands.**

	Substances Tested			
Category	**Lipids**	**Acid MPS**	**Neutral MPS**	**Aminated Substances**
Mature	−	+ + +	+ +	+
Large immature	−	+ +	+ +	+
Medium immature	*	+ +	+ +	+
Small immature	*	+ +	+ +	+

−, negative; +, weakly positive; + +, positive; + + +, strongly positive; *, test not possible.

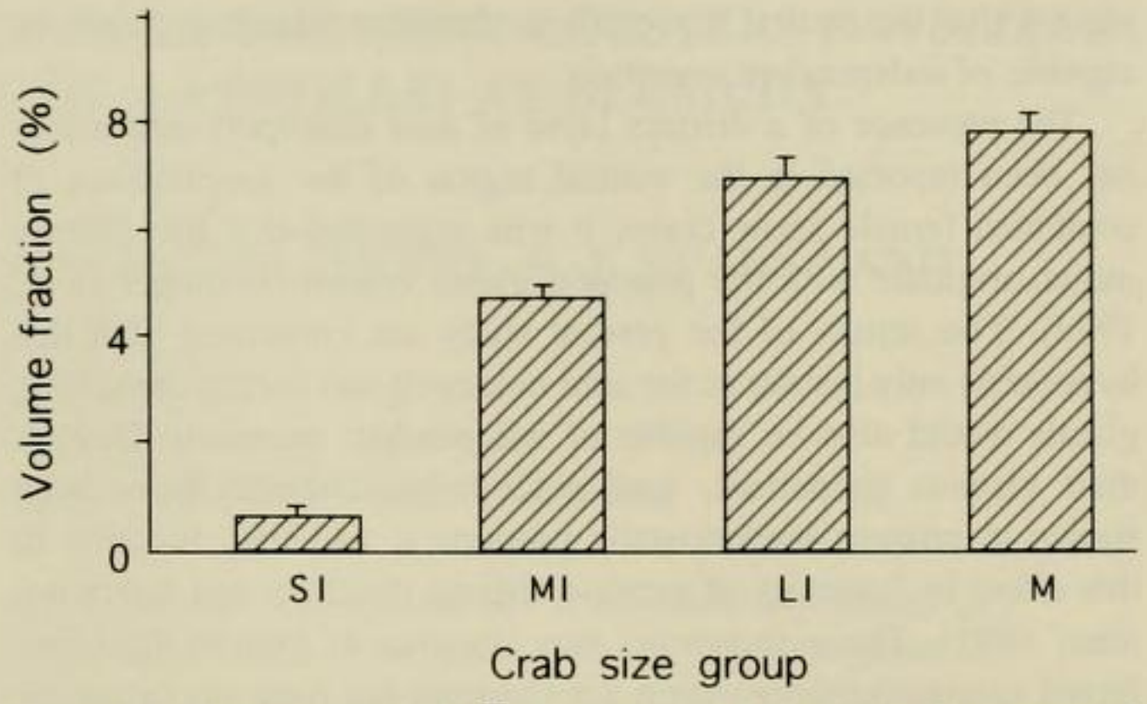

Figure 3. Volume fraction ($\bar{X}$ ± standard deviation) of tegumental glands within histological sections of gonopods of crabs from each size group. SI, small immature; MI, medium immature; LI large immature; M, mature.

most appropriate, in order to fully elucidate the development and constitution of these glands.

The continuity of the gland secretions with the surface of the ejaculatory canal cuticle is accomplished via the cuticular pores. Histologically, their structure resembles that of the cuticular pores characteristic of the general crustacean exoskeleton (Halcrow 1993), with the notable exception of their much larger dimension (2–4 μm vs 0.1–0.2 μm). This large pore dimension is consistent with a function in rapid secretion of large amounts of fluid. The cuticular pores appear only after the first glands and duct system have differentiated, several instars after metamorphosis, again underscoring the link with reproduction rather than cuticle hardening or maintenance.

The sampling carried out in the present study precluded observations of a seasonal nature, in the event of seasonal cycles in gland contents. Although highly desirable, such sampling is extremely difficult for the population studied, due to weather conditions and ice cover from November through May. Moreover, the stereological results clearly demonstrate a close relationship between maturity and gland volume fraction, regardless of sampling date. We would expect seasonal differences, if present, to consist of variations in the amount and type of secretions in the glands. Studies now in progress address these questions in relation to the adult reproductive cycle in more accessible populations and in other crab taxa.

Gonopod Tegumental Glands and Maturity Criteria

Maturity in male snow crabs may involve several aspects: morphometric, physiological, and functional (Conan and Comeau 1986, Comeau and Conan 1989, Claxton et al. 1994). There is disagreement, however, regarding the means of assessing maturity; this problem is related to and compounded by the lack of consensus on the existence of a male terminal moult in this genus, and whether the maturity moult is the terminal moult (Dawe et al. 1991, Paul 1992, Sainte-Marie and Hazel 1992). The stereological results of the present study show that gonopod tegumental glands continue to develop through all size groups to the morphometrically mature crabs. A stereological study involving a statistically appropriate number of individuals might usefully be undertaken, in order to determine whether the volume fraction of the gonopod tegumental glands could be used as a maturity criterion in this debate, in conjunction with the present morphometric technique and vas deferens weights (Comeau and Conan 1989).

Possible Roles of the Gonopod Glands

The histochemical data of the present study show that the main, if not exclusive, constituents of the secretions are mucopolysaccharides. The foremost biological properties of mucopolysaccharides are related to their viscosities. The *C. opilio* gonopod gland system comprises glands with exclusively neutral secretions, exclusively acid secretions, and mixed secretions. Increasing mucus viscosity is associated with a corresponding increase in the proportion of acid mucopolysaccharides (Grenon and Walker 1980, Moreno et al. 1982); this is supported by visual observations of mucus behaviour on surfaces known to secrete specific MPS types (Beninger et al. 1992, 1993). Neutral mucopolysaccharides are thus very fluid, and they may be used to reduce the viscosity of more highly viscous mucopolysaccharides (St-Jean 1993). The matrix of the vas deferens is composed of poorly mixed acid and neutral mucopolysaccharides, and is thus of intermediate viscosity. The seminal secretions and spermatophores must be forced through the narrow ejaculatory canal (diameter approx. 50 μm) (Beninger et al. 1991) during copulation; hence a reduction in their viscosity would facilitate spermatophore transfer, and may even be a necessary condition for such transfer. Such a process would

Figure 2. Histological sections of *Chionoecetes opilio* first gonopod. Alcian blue - PAS stain. 2.1. Small immature individual (CW = 28.5 mm). Note paucity of glands (G) and absence of pores in cuticle (CU) of ejaculatory canal (EC). 2.2. Medium immature individual (CW = 53.6 mm). Note increased density of glands (G) compared to preceding small immature specimen (ratio of 2.1:1 compared to Figure 2.1, after standardization of surface areas). DN, extensive duct network; P, cuticular pores, often accompanied by proximal dilatations (DL); LCT, loose connective tissue. 2.3. Detail of cuticle (CU) and duct network (DN) of same individual. Note penetration of pores (P) into cuticle, and granular substances within pores, similar to that in duct network (arrowheads). 2.4. Composite micrograph detail of same individual, showing pathway from gland (G) via duct (D) to duct network (DN) and cuticular pores (P). Note proximal dilatation (DL) of pore, and presence of granular substances at all levels of pathway (arrowheads). CU, cuticle.

require that the neutral mucopolysaccharide-containing glands be capable of independent secretion.

The presence of a distinct layer of acid mucopolysaccharides has been reported in the ventral region of the spermatheca of copulated female snow crabs; it was suggested that this feature might originate with the gonopod gland system (Beninger et al. 1993). The results of the present study are consistent with this hypothesis only insofar as the acid mucopolysaccharide-containing glands would also be capable of independent secretion. Besides their viscous properties, acid mucopolysaccharides have been shown to possess bacteriostatic properties, and may function to this effect in 2 species of penaeid shrimp (Sasikila and Subramoniam 1987). These secretions may thus act to protect the transferred spermatophores within the spermatheca from opportunistic microbes. Further research is needed to verify this hypothesis.

Mixed-secretion, intermediate viscosity mucopolysaccharides are ideal for lubrification. The specific distribution of the gland region within the first gonopod corresponds to the insertion of the second gonopod, which acts as a piston to force seminal fluids from the penis along the ejaculatory canal of the first gonopod (Beninger et al. 1991); lubrification would facilitate this movement and reduce the risk of damage to the cuticle of the ejaculatory canal. The above-mentioned functional possibilities are not mutually exclusive, but do imply some degree of differential control. Future studies might explore these avenues.

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