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Glyconjugates in epidermal, branchial and digestive mucous cells and gastric glands of gilthead sea bream, *Sparus aurata*, Senegal sole, *Solea senegalensis* and Siberian sturgeon, *Acipenser baeri* development

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SUMMARY

Epidermal, branchial and digestive mucous cells, and the gastric glands of larvae/postlarvae (from hatching until 45 days posthatching) of three fish species (two teleostean and a chondrostean) were investigated using conventional histochemical methods (periodic acid schiff –PAS-, diastase-PAS; alcian blue pH 0.5, 1 and 2.5) in order to distinguish neutral and acidic (carboxylated and sulphated) glycoconjugates, as well as bromophenol blue reaction for identification of proteins. Additionally, the presence and distribution of sugar residues in the oligosaccharide side chains of glycoconjugates were investigated using horseradish peroxidase (HPR)-conjugated lectins (Con A, DBA, WGA and UEA-I). Most mucous cells (digestive, epidermal and branchial) of Siberian sturgeon, *Acipenser baeri*, sea bream, *Sparus aurata* and Senegal sole, *Solea senegalensis* larvae were PAS- and alcian blue- (pH 2.5 and 0.5) positive, with small variations between organs/tissues and species. Bromophenol blue reaction (general proteins) was positive in a minority of the mucous cells, usually in those cells which were PAS-negative. Proteins rich in sulphydryl (-SH) and/or disulphide (-S-S-) groups related with the glycoprotein nature of the glycoconjugates present in mucous cells were also observed. Epidermal, branchial and digestive mucous cells of all studied larvae did not contain glycogen or lipids.

Con A lectin staining was negative in all mucous cells types of sea bream and sole, but oesophageal mucous cell of sturgeon were reactive to different lectin reactions, suggesting the presence of mannose –Man- and/or glucose –Glc-, L-fucose –Fuc- ; N-acetyl-D-galactosamine -GalNAc-, as well as N-acetyl-D-glucosamine- GlcNAc - and/or sialic acid –NANA- residues. Digestive mucous cells of all studied larvae were positive to WGA and DBA lectins. Epidermal and branchial mucous cells of sea bream and sole were Con A, DBA and UEA-I unreactive. However, mucous cells of sturgeon larvae were stained with UEA-I lectin.

Gastric glands appear very early in sturgeon stomach larvae development (between 5-6 days posthatching) but rather late (around 40 days) during the ontogeny of sole and sea bream larvae. These glands contain neutral glycoproteins with Man and/or Glc, Fuc, GlcNAc- and/or sialic acid and rich in GalNAc- sugar residues, as well as proteins moderately rich in arginine, and others particularly rich in tyrosine and tryptophan .

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INTRODUCTION

The histomorphological development of gilthead sea bream, *Sparus aurata*, Senegal sole, *Solea senegalensis* and Siberian sturgeon, *Acipenser baeri* larvae and juveniles was studied previously by Sarasquete *et al.* (1993, 1995, 1996, 1998); Domeneghini *et al.* (1998), Ribeiro *et al.* (1999), Gisbert *et al.* (1998, 1999) and Vieira (2000). In the present study, we show a histochemical comparative study of mucous cells of skin, gills and digestive tract (oesophagus and intestine), before and during gastric gland development (until 45 days posthatching) in these species (two teleostean fish: Senegal sole and sea bream and a chondrostean species: Siberian sturgeon), these species present very different developmental patterns (yolk-sac exhaustion, metamorphosis, digestive, branchial and epidermal development, etc.), as well as different life styles and feeding habits.

In fish, the mucous or goblet cells produce the mucins -glycoconjugate content- which covers the body, and these cells are distributed over the skin, on the gills and line the digestive tract (Roberts, 1978; Gona, 1979; Groman, 1982). In mammals, glycoconjugates exert a large variety of functions: mechanical, antimicrobial, antiviral, osmotic, ionic transport, etc. (Allen, 1981). Gut mucosubstances may also exert an osmotic function in fish (Smith, 1989). Mucins, the main constituents of mucus, are high molecular weight glycoproteins. About 50% of their dry weight can consist of carbohydrate chains (Strous and Dekker, 1992).

Digestive efficiency in fish depends on the proteolytic action of pancreatic juice, goblet cell secretions and intracellular digestion (Kapoor *et al.*, 1975). Glycoconjugates containing neutral sugars protect the mucosa against proteolytic degradation, and may also have a buffering effect on the high acidity of the stomach contents (Smith, 1989; Scocco *et al.*, 1996). On the other hand, the presence of neutral mucins and alkaline phosphatase activity in the digestive epithelium of fish could be positively correlated with absorption and transport of macromolecules through membranes (Stroband *et al.*, 1979). To prevent physical and chemical damage to the gut lining, acid glycoproteins probably act as a lubricant to the fibre-rich material in the gut. They have also a role in the assimilation of nutrients from plants (Tibbets, 1997).

In fish, active ionoregulation begins in the early stages of larval development. Since gills and kidney are still poorly developed at these stages (until metamorphosis), the skin has been presumed to be the functional site of ionoregulation (Morrison, 1993). Instead of branchial respiration, fish larvae rely on cutaneous gas exchange. Even if larvae possessed morphologically differentiated gills, branchial respiration would be rather ineffective (Segner *et al.*, 1994).

Mucous cells and the composition of the mucins they produce are influenced by endogenous factors (sex, development stage, etc.) and exogenous factors, such as stress acid and infections (Ferguson *et al.*, 1992). Mucous secretion of skin and gills may be important in natural defense against parasites and pathogenic microorganisms (Fletcher, 1978). On the other hand, changes in the number and dimension of mucous cells of gills and skin can be an indicator of pathological or inflammatory processes induced by adverse environmental conditions (Lemoine and Olivereau, 1971; Wendelaar-Bonga and Lock, 1992; Ortiz *et al.*, 1999). The sialic acid content has been used to estimate the degree of skin mucification in fishes (Arillo *et al.*, 1979). Moreover, sialitation and sulphation of glycoproteins, the main component of the mucous cells, may be important for increasing the resistence of mucous to bacterial degradation (Rhodes *et al.*, 1985).

Comparison of the glycoconjugate composition of mucous secretion in larvae and adult fish reveals remarkable inter- and intra-specific differences (Madrid *et al.*, 1989**;** Illana, 1993; Pajak and Danguy, 1993; Sarasquete *et al.*, 1995; 1996; 1998; Burkhardt-Holm, 1997; Domeneghini *et al.*, 1998, 1999; Ribeiro *et al.*, 1999; Gisbert *et al.*, 1998, 1999; Arellano *et al.*, 1999). This variety can be attributed not only to different feeding habits, but possibly to different taxonomic position, development and biology (Reifel and Travill, 1977, 1978, 1979; Domeneghini *et al.*, 1998, 1999).

MATERIALS AND METHODS

Siberian sturgeon, *Acipenser baeri*, Senegal sole, *Solea senegalensis* and sea bream, *Sparus aurata* larvae/postlarvae, from hatching until day 45 posthatching were fixed in Bouin solution or in 10% v/v buffered formaldehyde (pH 7.2) and

embedded in paraffin. Histological sections of whole specimens of 5-7 μm thickness were stained with haematoxylin-eosin and haematoxylin-V.O.F (light green-orange G –acid fuchsin) according to Gutierrez (1990). The cytochemical tests for carbohydrates (PAS, diastase-PAS; alcian blue pH 0.5, 1 and 2.5, acetylation, saponification, chlorhydric hydrolysis, neuraminidase-type V from *Clostridium perfringens*, bacterial hyaluronidase from *Streptomices hyalurolyticus)*, as well as for glycoproteins (lectins), general proteins (bromophenol blue), proteins rich in lysine (ninhydrin-Schiff); proteins rich in tyrosine (Hg-sulphate-sulfuric acid-sodium nitrate); proteins rich in trytophan (P-dimethylaminobenzaldehyde), proteins rich in arginine (1,2 napthoquinone-4-sulphonic acid salt sodium); -SH and –S-S- (sulphydryl and disulphide groups; ferric ferricyanide-Fe III and thioglycollate reduction) and lipids (Oil Red 0 and Sudan black B in cryostat sections) used in this study are described by Pearse (1985) and Bancroft and Stevens (1990).

For the lectin analysis of glycoconjugates, sections were treated with 0.3% hydrogen peroxide for 10 minutes (to inhibit endogenous peroxidase) in Tris buffered saline (TBS) at pH 7.2. The sections were incubated for 30 minutes at room temperature in the presence of the following horseradish peroxidase-conjugated lectins (HPR-lectin conjugated) dissolved in TBS (20 μ/ml): ConA (mannose-Man-and/or glucose-Glc-), WGA (N-acetyl-D-glucosamine-GlcNAc- and/or sialic acid-NANA-), DBA (N-acetyl-D-galactosamine-GalNAc-) and UEA-I (L-Fucose –Fuc-). After three washes in TBS, peroxidase activity was visualized with TBS containing 0.05% 3,3´diaminobenzidine tetrahydrochloride and 0.015% hydrogen peroxide. Sections were washed in running tap water for 10 minutes, dehydrated, cleared and mounted in Eukitt.

Controls were: omission of the respective lectin; substitution of lectin-HPR conjugates by TBS and treatments with different enzymes (neuraminidase Type V –0.9 U/mg Prot-; β-galactosidase Grade VI –400 U/mg Prot-; α-mannosidase Type III –20 U/mg Prot -and β-N-acetylglucosaminidase –0.25 U/mg Prot-: L-fucosidase –20U/mg Prot).

Lectins and enzymes were purchased from Sigma Chemical Co., St. Louis, MO, USA.

RESULTS

The histochemical characteristics of carbohydrates/glycoconjugates, proteins and lipids in gastric glands of stomach, intestinal enterocytes and mucous cell secretions of digestive tract (oesophagus and intestine), gills and skin of the Siberian sturgeon, *Acipenser baeri*, sea bream, *Sparus aurata* and Senegal sole, *Solea senegalensis* during different stages of larvae/postlarvae development are shown in Tables I-III. Some of these histochemical results are shown in Figures 1-4.

The species studied presented some histochemical differences in oesophageal mucous cell glycoconjugate content (Table I). The secretory cells of the distal oesophageal zone, close to the undeveloped stomach or to the functional stomach, secreted large amounts of neutral glycoconjugates, and those cells of the proximal pharynx-oesophageal zone produced, in addition to a minor component of neutral glycoconjugates, a great quantity of acidic glycoconjugates, sulphated and carboxylated with sialic acid substituted at C8.

The gastric mucosa of sea bream, sole and sturgeon secreted a large quantity of neutral glycoconjugates, together with small amounts of sialoglycoconjugates. Sialosulphomucins were not observed in gastric glands, which contained neutral glycoproteins with GalNAc, Fuc, GlcNAc and Man and/or Glc sugar residues. In all studied species, when gastric glands were developed, they were strongly positive to general and specific protein reagents, such as stains for tyrosine, arginine, lysine, tryptophan, and –SH and –S-S- groups (Table II).

During the endogenous feeding period of sea bream, sole and sturgeon larvae, the epithelium that lines the developing gut secreted a moderate quantity of neutral glycoconjugates. Most intestinal mucous cells presented a combination of neutral and acidic glycoproteins, while a few of them contained mainly acidic or neutral glycoproteins (Table I). These acidic mucins were composed by both sialomucins and sulphoglycoconjugates.

While epidermal mucous cells of sturgeon, sole and sea bream larvae (and those of gills in sturgeon) appear very early during larval development (at 3-4 days post hatching), branchial mucous cells are developed around 40 days posthatching in sea bream and sole. Initially, epidermal mucous cells secreted neutral mucins (PAS and diastase-PAS pos-

Table I
Proteins and glycoconjugates in mucous cells of oesophagus and intestine of Siberian sturgeon, *Acipenser baeri*, Senegal sole, *Solea senegalensis* and sea bream, *Sparus aurata* larvae

	Oesophagus			Intestine		
	Sturgeon	**Solea**	**sea bream**	**Sturgeon**	**Solea**	**sea bream**
Neutral Glycoproteins (P-A)	2-3	1-2	2-3	3	2	0-3
Glycogen	0	0	0	0	0	0
Sialoglycoproteins (P-A)	1-3	1-2	1-2	3	3	3
Sulphated-glycoproteins (P-A)	1-3	1-2	1-3	3	0-1	3
Man/Glc	1	0	0	0	0	0
Fuc	0-1	0	0	2-3	0	0
GlcNAc/Nana	0-1	2	3	2-3	1-3	1-2
GalNAc	0-1	0-2	1-3	3	1	2
Lipids	0	0	0	0	0	0
Proteins	2	0-1	0-1	2	1	1
-S-S- groups	3	0-1	0-1	2	1	0-1

Intensity of reactions: 0: negative; 1: weak; 2: moderate and 3 strong histochemical reactivity. A: anterior oesophageal portion; P: posterior oesophageal portion.

itivity), but progressively during larval development, some mucous cells were strongly stained with Alcian Blue pH 0.5 and 2.5, and especially with alcian blue pH 1 (sulphated groups weakly ionized). When alcian blue pH 2.5-PAS reaction was performed, most mucous cells stained blue (carboxylated groups); some cells stained red (neutral mucins) and a few mucous cells stained purple, indicating a combination of neutral and acidic mucins. PAS reactivity was weakly increased after saponification, suggesting the presence of acetylated sialic acids. Moreover, sialic acid was also evidenced in epidermal mucous cells with WGA-lectin, because a slight decrease in WGA staining and a decreased alcianophilia (alcian blue pH 2.5) after neuraminidase treatment were observed in these mucous cells. Epidermal and branchial mucous cells of sea bream and sole larvae were ConA, UEA-I and DBA unreactive. However, Fuc (UEA-I lectin) residues were detected in digestive, epidermal and branchial mucous cells of sturgeon (Table III).

DISCUSSION

According to the histological/histochemical characteristics, oesophageal mucous cells of Siberian sturgeon, *Acipenser baeri* larvae seemed to be fully developed and functional between 7-8 days posthatching (2 days before the onset of exogenous feeding). This fact (Gisbert *et al.*, 1998, 1999) differs from different teleostean fish larvae, such as turbot, *Scophthalmus maximus* (Cousin and Baudin-Laurencin 1985); Dover sole, *Solea solea* (Boulhic and Gabaudan, 1992); sea bream, *Sparus aurata* (Sarasquete *et al.*, 1995; Domeneghini *et al.*, 1998) and Senegal sole, *Solea senegalensis* (Sarasquete *et al.*, 1996; Ribeiro *et al.*, 1999), whose oesophageal mucous cells appear and differentiate later in development.

All the studied species presented some histochemical differences in oesophageal mucous cell glycoconjugate content considering that the secretory cells of the distal oesophageal zone secrete large amounts of neutral glycoconjugates. However, the secretory cells of the proximal pharynx-oesophageal zone produced, in addition to a minor component of neutral glycoconjugates, a great quantity of acidic glycoconjugates, sulphated and carboxylated with sialic acid substituted at C8. These results were shown in Senegal sole larvae, and previously observed in sea bream (Domeneghini *et al.*, 1998) and in Siberian sturgeon (Gisbert *et al.*, 1999). The acidic glycoconjugates present in white sturgeon, *Acipenser transmontanus* oesophagus, were shown to be of the sialylated type, so that the mucosal secretion is likely rather fluid (Domeneghini *et al.*, 1999). A combination of mucosubstances (neutral and acidic) was described as a mechanism that allows the alimentary canal of young fish to respond

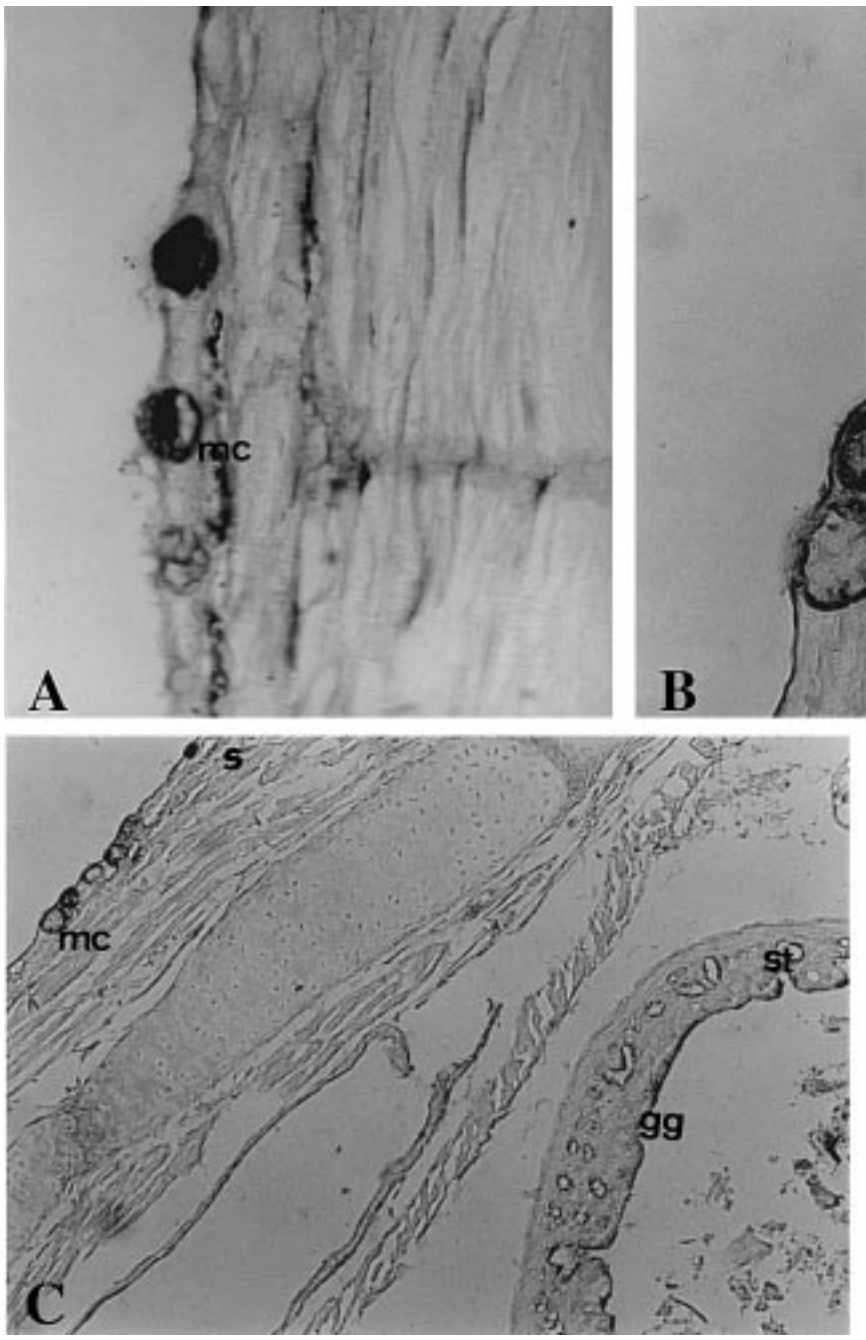

Fig. 1 - Histological sections of Siberian sturgeon, *Acipenser baeri* larvae. **A)** Acid and neutral glycoproteins in epidermal mucous cells of larvae at 9 days post hatching). Alcian-blue 2.5-PAS reaction. x 400. **B)** Glycoconjugates containing GlcNAc and/or sialic acid residues in epidermal mucous cells of larvae at 15 days post hatching. WGA-lectin. x400. **C)** Presence of glycoconjugates containing GlcNAc and/or sialic acid residues in epidermal mucous cells and gastric glands of larvae (15 days post hatching). WGA-lectin. x100. **gg: gastric glands; mc: mucous cells; s: skin; st: stomach.**

to different changes in environmental conditions early on. The large number of oesophageal mucous cells and the large amount of mucins secreted, however, may not be explicable simply as a lubricant (Reifel and Travill, 1977; Scocco *et al.*, 1998). As was reported by Zimmer *et al.* (1992), the presence of sialic acid residues prevents viruses from recognising their receptor determinants, and also protects the mucosa from attack by sialidase produced by bacteria. Furthermore, neutral glycoconjugates secreted by mucous cells are reputed to cooperate in the enzymatic digestion of food and in transforming it into chyme, as well as in absorptive functions in other regions of the alimentary canal (Grau *et al.*, 1992; Murray *et al.*, 1994).

On the other hand, it is important to note that buccal

Table II
Glycoconjugates, proteins and lipids in intestinal enterocytes and in gastric glands of Siberian sturgeon, *Acipester baeri*, Senegal sole, *Solea senegalensis* and sea bream, *Sparus aurata* larvae

	Cytoplasm/Brush Border			**Gastric Glands**		
	Sturgeon	**Solea**	**Seabream**	**Sturgeon**	**Solea**	**Seabream**
Neutral Glycoproteins	1/3	1/ 2	1/ 2	3	1	1
Glycogen	2/1	2/1	1/1	0	0	0
Sialo-glycoproteins.	1/1	0-3	1/1	0	0	0
Sulphated-glycoproteins	0/0	3/0	1/0	0	0	0
Man/Glc	1/1	2/2	1/1	1	1	1
Fuc	2/2	2/2	1/1	1	1	1
GlcNAc/Nana	0/3	1/ 2	2/3	2	1	1
GalNAc	2/3	1/1	2/2	1	1	1
Lipids	2/0	2/2	2/0	0-1	0-1	0-1
Proteins	2/3	2/3	2/3	2-3	2-3	2-3
Arginine	2/2	1/ 2	1/ 2	2-3	2-3	2-3
Tyrosine	2/2	1/ 2	1/ 2	3	3	3
Tryptophan	0/0	1/ 2	1/ 2	3	3	3
Lysine	1/1	2/3	1/ 2	1	1	1
-SH (sulphydryl groups	1/1	1/ 2	1/1	1	1	1
-S-S- (disulphyde groups)	1/ 2	2/3	2/2	2	2	2-3

Intensity of reactions: 0: negative; 1: weak; 2: moderate and 3 strong histochemical reactivity

Table III
Proteins and glycoconjugates in mucous cells of skin and gills of Siberian sturgeon, *Acipester baeri*, Senegal sole, *Solea senegalensis* and sea bream, *Sparus aurata* larvae

	Skin			**Gills**		
	Sturgeon	**Solea**	**Seabream**	**Sturgeon**	**Solea**	**Seabream**
NeutralGlycoproteins	1-3	1	1	2-3	2	2
Glycogen	0	0	0	0	0	0
Sialoglycoproteins	3	2	3	3	2	2
Sulphated- glycoproteins	3	1	3	0-1	3	3
Man/ Glc	0	0	0	0	0	0
Fuc	1	0	0	1	0	0
GlcNAc/Nana	1-3	1-3	3	1	1-3	1-3
GalNAc	0	0	0	0	0	0
Lipids	0	0	0	0	0	0
Proteins	1	0	2	1	2	2
-S-S- groups	2	1	0	2	1	1

Intensity of reactions: 0: negative; 1: weak; 2: moderate and 3 strong histochemical reactivity.

salivary glands are normally lacking in fish, so that the oesophageal mucous cells might execute, among other things, the functions of mammalian saliva, i.e. protecting the mucosa of the entire alimentary canal (Scocco *et al.*, 1998). In sturgeon, oesophageal mucous cells were Con A (Man and/or Glc residues) and WGA (GlcNAc and/or NANA) reactive. However, oesophageal mucous cells of sea bream and sole, as well mucous cells of skin, gills and intestine of all studied species were ConA unreactive The presence

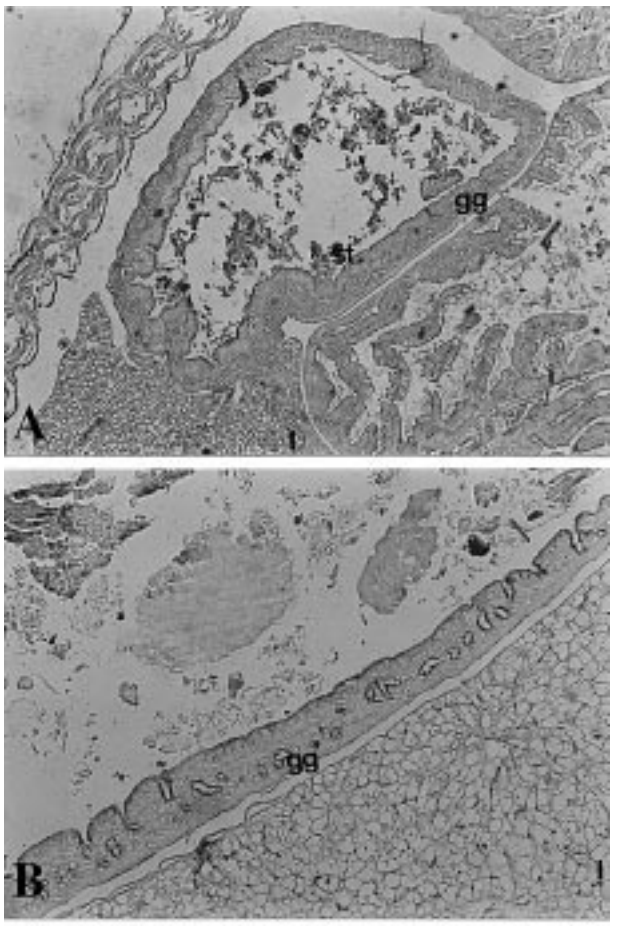

Fig. 2 - Histological sections of Siberian sturgeon, *Acipenser baeri* larvae. **A)** Gastric glands and intestinal mucosa containing Fuc residues. Larvae at 15 days post hatching. UEA-lectin x100. **B)** Presence of Man and/or Glc residues in glycoconjugates of the gastric glands. Larvae at 15 days post hatching. Con A-lectin. x 100. **C)** Glycoconjugates containing GalNac residues in gastric glands of larvae at 15 days post hatching. DBA-lectin. x100. **gg: gastric glands; l: liver; i: intestine: st: stomach.**

of WGA, Con A , UEA-I and DBA reactive granules in the epithelium and goblet cells of the Siberian sturgeon esophagus supports the concept that carbohydrate absortion occurs in this area, as was demostrated for other digestive sections (intestine and stomach) of adult fishes (Reifel and Travil, 1978; Scherbina *et al.*, 1978; Madrid *et al.*, 1989; Grau *et al.*, 1992; Sarasquete *et al.*, 1996). The absence of neutral mucosubstances and the presence of acidic glycoproteins at the oesophageal level may indicate a secretory rather than an absorptive function in fish (Kapoor *et al.*, 1975; Grau *et al.*, 1992),

Stomach

Developed gastric glands were detected very early, at 8-9 days posthatching, in Siberian sturgeon larvae development (Gisbert *et al.*, 1998, 1999) and between 35 and 45 days posthatching in sea bream (Domeneghini *et al.*, 1998) and Senegal sole (Vieira, 2000). These results have been related to metamorphosis in these species (Buddington and Christofferson, 1985; Sarasquete *et al.*, 1995; Gisbert *et al.*, 1998; Vieira, 2000). Sialosulphomucins were not observed in gastric glands of *S. senegalensis, S. aurata* and *A. baeri*, which contain neutral glycoproteins with GalNAc, Fuc, GlcNAc and Man and/or Glc sugar residues. However, glandular cells of *S. aurata* and *A. transmontanus* stomach were unreactive to the histochemical tests for glycoconjugates (Domeneghini *et al.*, 1998, 1999). According to Domeneghini *et al.* (1998), GalNAc and GlcNAc residues could be identified in the sea bream gastric glands for a limited larval period only, but were no longer detectable in 100-day-old fish, probably because they were then in a sub-terminal position in the oligosaccharide branch. Only when the juvenile stage was reached did the adherent mucus gel appear to be composed of glycoconjugates containing N-acetyl-galactosamine, α-D-mannose, β-D-galactose, L-fucose and N-acetyl-glucosamine, in a pattern similar to that seen in sea bream adult fish (Domeneghini *et al.*, 1998). These sugars probably occupy a terminal position in the glycoconjugates only when they are secreted to form the adherent mucus gel of the gastric mucosa.

The gastric mucosa of *S. aurata, A. transmontanus* (Domeneghini *et al.*, 1998, 1999), *A. baeri* and *S. senegalensis* secretes a large quantity of neutral glycoconjugates, together with small amounts of sialoglycoconjugates. Secretion of neutral glycoconju-

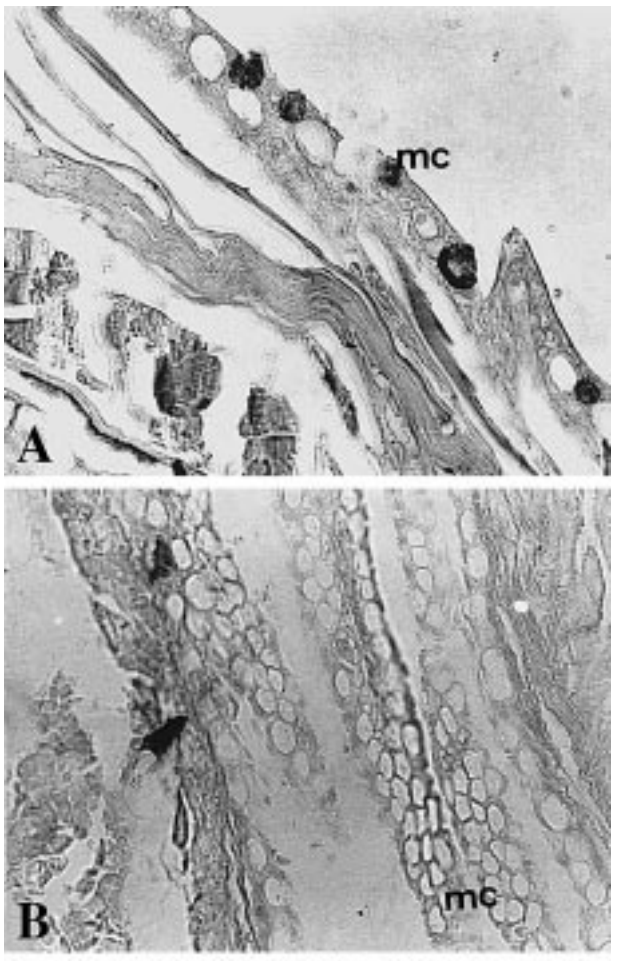

Fig. 3 - Histological sections of Senegales sole, *Solea senegalensis* and *Sparus aurata* larvae. **A)** Presence of glycoconjugates containing GlcNAc and/or sialic acid in epidermal mucous cells of *Solea senegalensis* larvae (15 days post hatching). WGA-lectin. x400. **B and C)** Absence of Man and/or Glc residues in glycoconjugates present in oesophageal mucous cells of *Solea senegalensis* **(A)** and *Sparus aurata* **(B)** (larvae at 15 days post hatching). ConA-lectin. x400. **mc: mucous cells.**

gates containing sugar residues has been observed in gastric glands of different fish species (Gutiérrez *et al.*, 1986; Ferraris *et al.*, 1987; Domeneghini *et al.*, 1998; Gisbert *et al.*, 1998, 1999), and may serve to protect the epithelium of stomach from autodigestion processes caused by HCl and enzymes produced in gastric glands (Ferraris *et al.*, 1987). In fish, these glands are composed of one cell type, named "oxyntopeptic cell" (Reifel and Travill, 1978; Elbal and Agulleiro, 1986). These authors pointed out that the positive-PAS reaction seen on the surface of gastric epithelial cells resembles that seen in the striated border of intestinal enterocytes. This may indicate nutrient absorption occurring in the stomach. In fact, the presence of neutral mucins in the stomach has been related to the absorption of easily digestable substances such as disaccharides and short-chain fatty acids (Grau *et al.*, 1992)**.** Sulphated glycoproteins were negative in gastric glands of all studied species, but they were present in the stomach of a variety of other fish species (Reifel and Travill, 1978; Grau *et al.*, 1992). Spicer and Schulte (1992) speculated that because of their known antipeptic activity, sulphomucins may be able to form a complex with pepsin, thereby stabilizing or buffering the enzyme.

The presence of proteins rich in tyrosine, arginine and tryptophan in developed gastric glands of all studied species suggests the presence of enzymatic precursors such as pepsinogen or digestive enzymes, as has been observed for different fish species (Medeiros *et al.*, 1970 a,b; Gutiérrez *et al.*, 1986; Grau *et al.*, 1992; Gisbert *et al.*, 1999; Douglas *et al.*, 1999; Vieira, 2000).

Intestine

The presence of neutral glycoconjugates in the epithelium that lines the developing gut of different fish larvae suggests a secretory function of the intestinal epithelium (Domeneghini *et al.*, 1998). The presence of neutral mucins and alkaline phosphatase activity in the brush border of intestinal epithelium has been positively correlated with absorption and transport of macromolecules through membranes (Stroband *et al.*, 1979). Considering that the distal part of the gut of most fish species is capable of ingesting and digesting proteins via a pinocytotic pathway (Segner *et al.*, 1994), and that this part shows maximal nutrient uptake capacity in the sturgeon (Buddington and Doroshow, 1986 a, b), Domeneghini *et al.* (1999) assumed that the muco-

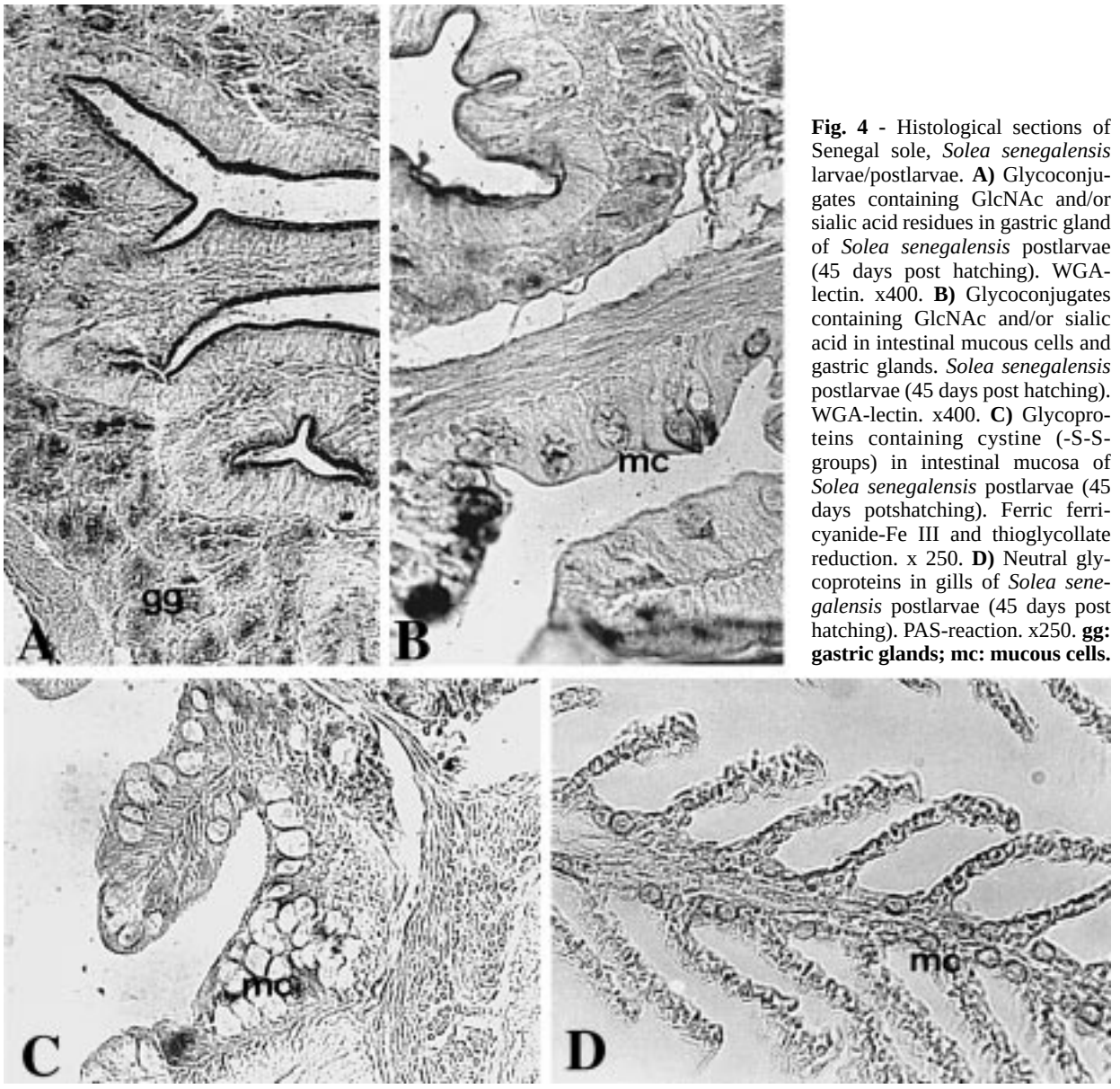

Fig. 4 - Histological sections of Senegal sole, *Solea senegalensis* larvae/postlarvae. **A)** Glycoconjugates containing GlcNAc and/or sialic acid residues in gastric gland of *Solea senegalensis* postlarvae (45 days post hatching). WGA-lectin. x400. **B)** Glycoconjugates containing GlcNAc and/or sialic acid in intestinal mucous cells and gastric glands. *Solea senegalensis* postlarvae (45 days post hatching). WGA-lectin. x400. **C)** Glycoproteins containing cystine (-S-S-groups) in intestinal mucosa of *Solea senegalensis* postlarvae (45 days potshatching). Ferric ferricyanide-Fe III and thioglycollate reduction. x 250. **D)** Neutral glycoproteins in gills of *Solea senegalensis* postlarvae (45 days post hatching). PAS-reaction. x250. **gg: gastric glands; mc: mucous cells.**

subtances, especially the sulphated ones, may regulate the transfer of protein or protein fragments.

The intestinal mucosa of sturgeon, *A. transmontanus* was organized in folds, containing numerous mucous cells which synthesized neutral or acidic glycoconjugates, the latter either of the sialylated or sulphated type. The sulphoglycoconjugates were more abundant in the mucous cells of the distal intestinal tract (Domeneghini *et al.*, 1999). Similar results were observed in *A. baeri*. Most intestinal mucous cells of sea bream, sole and sturgeon larvae presented a combination of neutral and acidic glycoproteins, while a few of them contained mainly acidic or neutral glycoproteins. Variability in staining within a given cell could be attributed to a temporal sequence in the biosynthesis of mucins (Harrison *et al.*, 1987). The coexistence of neutral and acid glycoconjugates may be an indication of mucous cell differentiation with maturity (Elbal and Agulleiro, 1986; Murray *et al.*, 1996). Intestinal mucous cells of all studied species were negative with the lipid techniques used, such as was observed in other fish species (Grau *et al.*, 1992; Sarasquete *et al.*, 1995; Ribeiro *et al.*, 1999; Arel-

lano *et al.*, 1999). Some mucous cells of sole, sea bream and sturgeon were only positive to the PAS reaction, while other cells were weakly positive to bromophenol blue (proteins). With alcian blue pH 2.5-PAS double-staining, numerous cells were stained in red and/or blue and a few cells staining purple. Biosynthesis of mucin glycoconjugates includes at least two post-transcriptional modifications of the secretory protein: firstly glycosylation of the proteins, followed by modification of the sugar moiety (Phelps, 1978). According to Els and Hennerberg (1990), cells not staining with PAS contain only proteins. PAS-positive mucous cells might represent an early cell developmental stage, when the cells are producing mainly glycoproteins. Mucins stain with alcian blue (pH 2.5) when the glycoproteins are carboxylated, and the presence of sulphated glycoproteins (AB pH 0.5 and 1) coincides with the stage when sulphated groups are conjugated to the glycoprotein. Similar observations were made by Arellano *et al.* (1999) in relation to glycoconjugate content of intestinal mucous cells of *Solea senegalensis* adult specimens.

The goblet cells of the intestinal mucosa of Siberian sturgeon, Senegal sole and sea bream larvae were WGA and DBA reactive, suggesting the presence of GalNAc and GlcNAc sugar residues. However, according to Domeneghini *et al.* (1998), these lectins do not bind to the intestinal mucosa of fishes younger than 70 days of age**.**

Gills and Skin

While epidermal mucous cells of all studied species and those of gills in Siberian sturgeon appear very early during larval development (around 3-4 days posthatching), branchial mucous cells appear around 40 days posthatching in sea bream and sole.

Mucous secretory products of skin, gills and digestive tract (oesophagus and intestine) do not contain lipids or glycogen; the general protein reaction (bromophenol blue) was negative in some cells and positive in others. Most mucous cells contain disulphide groups (-S-S-), supporting the glycoprotein nature of their secretions, as was suggested by Arellano *et al.* (1999).

The mucin content of mucous cells (digestive tract and/or skin and/or gills) of different fish species, larvae and adults, did not show any affinity towards LTA, UEA-I, LCA and Con A lectins (Madrid *et al.* 1989; Pajak and Danguy, 1993; Burkhardt-Holm, 1997; Domeneghini *et al.*, 1998; Sarasquete *et al.*, 1998; Ribeiro *et al.*, 1999; Gisbert *et al.*, 1999), suggesting the absence not only of Man and/or Glc residues, but also of Fuc in the carbohydrates involved. However, Con A (Man/Glc residues) reactivity occurred in epidermal mucous cells of *Halobatrachus didactylus* and *Anguilla anguilla* adult specimens (Illana *et al.*, 1993), as well as in oesophageal mucous cells of Siberian sturgeon larvae (Gisbert *et al.*, 1999). In rainbow trout, *Oncorhynchus mykiss*, UEA-I (L-Fuc residues) binds with differing intensities to mucous cells at the tip of the primary and secondary lamellae of gills (Burkhardt-Holm 1997). According to this author, D (+) galactose and N-acetyl-D-galactosamine sugar residues were detected in glycoconjugates of epidermal and branchial mucous cells of rainbow trout, but epidermal mucous cells were ConA and UEA unreactive.

Future studies are planned to examine the influence of environmental contaminants (detergents, heavy metals, organic lipophilic pollutants, etc) on the lectin-binding pattern, because it is known or supposed that numerous contaminants can produce changes in the pH of the water, and these variations could influence the lectin-binding pattern, as well as the mucous secretion-rate of epidermal and branchial fish mucous cells; thus, the histochemical methods could provide a good tool or indicator for environmental field studies.

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