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A Study on the Epidermal Structure and Mucosubstance Histochemistry of Spotty Belly, Devil Stinger, Stone Fish, Cubed Snailfish, and Japanese Bluefish

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Abstract: Structure and mucosubstance histochemistry in the epidermis of five teleostean species, i. e., spotty belly, *Agrammus agrammus*, devil stinger, *Inimicus japonicus*, stone fish, *Erosa erosa*, cubed snailfish, *Liparis tessellatus*, and Japanese bluefish, *Scombrops boops* were investigated. The epidermis of five species studied is composed of three layers: superficial, middle, and basal layer. The superficial layer is comprised of rather flattened cells. Mucous cells, the type commonly found in fishes are completely lacking in the epidermis of devil stinger, stone fish, and cubed snailfish. The epidermis of devil stinger and stone fish have multicellular glands which do not have mucosubstances. The skin surface of them is covered with mucous layer. The superficial cells in the epidermis of devil stinger, stone fish, and cubed snailfish are mucus-secreting cells. The composition and the amount of the mucosubstances vary in species and body regions. The mucous layer on the skin surface and superficial epidermal cells of devil stinger contain a mixture of neutral and acidic (sulfated and non-sulfated) mucosubstances. In stone fish, the mucous layer has acidic (sulfated and non-sulfated) mucin and the superficial epidermal cells contain neutral mucin. In cubed snailfish, the type of epidermal mucosubstances is identified as a mixture of neutral and acidic (non-sulfated) mucin. The mucous cells of the epidermis in spotty belly and Japanese bluefish contain neutral mucin.

Key words: epidermis, fishes, mucosubstance, histochemistry

The primary function of the epidermis is protection against environmental hazards. In fish, this function is generally attributed to the gland cells, such as mucous cells and club cells, secreting their contents on surface. Secretory mucins are the major constituents of the mucus layer in which several biochemical compounds have been identified; like lysozyme, antimicrobial peptides, antibodies, C-reactive protein, and hemolysin (Alexander and Ingram, 1992; Shephard, 1994; Cole et al., 1997). A number of functions have been ascribed to the mucous layer, such as protection against mechanical injury (Pickering and Richards, 1980), friction reducing properties (Rosen and Conford, 1971), and possibly a role in ionoregulation (Handy et al., 1989). The epithelial mucous coat of fish also forms the primary barrier against infection (Pickering and Macey, 1977) in an external environment that harbours a multitude of potentially harmful or opportunistic microorganisms (Olafsen, 1995).

Previous histochemical studies on the mucosubstances in the epidermis of fishes have shown that the properties of the substances are diversified in species and inhabitants (Kazuyori and Motoyoshi, 1975; Singh and Mittal, 1990; Mittal et al., 1994; Lee and Kim, 1999; Lee et al., 2000; Kim et al., 2002; Jeong and Jo, 2007). Acidic sulfated mucopolysaccharides in mucous cells have reported in cutaneous respiratory fishes (Mittal and Munshi, 1971; Mittal and Banerjee, 1974; Mittal et al., 1980; Park and Kim, 2000 and 2007; Park et al., 2001).

This study is on the histological structure and histochemistry of the mucosubstances of the epidermis in five teleostean species, i. e., spotty belly, devil stinger, stone fish, cubed snailfish, and Japanese bluefish.

MATERIALS AND METHODS

Live adult specimens of spotty belly, *Agrammus agrammus*, devil stinger, *Inimicus japonicus*, stone fish, *Erosa erosa*, cubed snailfish, *Liparis tessellatus*, and Japanese bluefish, *Scombrops boops* were collected. Skin pieces (c. 5×8 mm^2) excised from the abdominal, thoracic and dorsal regions were fixed in 10% neutral phosphate buffered formaldehye.

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Paraffin sections were cut at 6 μm, and were stained with hematoxylin-eosin (H-E) and periodic acid Schiff (PAS) reaction (McManus, 1968).

For general histochemistry of mucosubstances, the following staining procedures were used: PAS reaction (McManus, 1968) to detect neutral mucosubstances, alcian blue (AB) staining at pH 2.5 (Spicer et al., 1967) to assess the nature of the acidic mucosubstances, AB at pH 1.0 (Lev and Spicer, 1964) to detect selective characterization of sulfated mucosubstances, and combined stainings with AB at pH 2.5-PAS (Mowry, 1963) to prove a composition of neutral and acidic mucosubstances, AB at pH 1.0-PAS (Lev and Spicer, 1964) to demonstrate a composition of neutral and sulfated mucosubstances, aldehyde fuchsin (AF) at pH 1.7-AB at pH 2.5 (Spicer and Meyer, 1960) to distinguish between sulfated and non-sulfated (sialyated) mucosubstances.

RESULTS

The histological structure of the skin in five teleostean species

The fundamental structure of the epidermis, which consists of three layer, the superficial, middle, and basal layer, is the same among the five teleostean species, although the thickness of the epidermis in three regions (abdominal, thoracic, and dorsal skin) is different.

The superficial layer is composed of epidermal cells which are arranged in about 3 to 6 rows. The superficial epidermal cells vary from cuboidal to flat in shape, are located in the epidermis surface. Cells in the middle layer, which are located between the superficial layer and basal layer, are large in size and irregular in shape. They occupy the largest portion of the epidermis in each of the five species. The basal layer consists of a single layer of columnar or polygonal cells, which are arranged in a single row on the basal membrane.

In devil stinger (Figs. 1C~1E) and stone fish (Figs. 1F and 1G), mucous layers on the surface of the epidermis are found. The superficial cells of devil stinger (Figs. 1C~1E), stone fish (Figs. 1F and 1G), and cubed snailfish (Figs. 1H and 1I) are mucus-producing cells.

Mucous cells commonly found in fishes are not found in any epidermis of three regions of devil stinger, stone fish, and cubed snailfish, although mucosubstances are found on the skin surface or in the epidermis of these species. In spotty belly (Figs. 1A and 1B) and Japanese bluefish (Figs. 1K and 1L), between epithelial cells, unicellular mucous cells are observed. Most mucous cells are spherical or some flask-shaped structures, but some in the abdomen have a large spherical body and a short narrow neck. The mucous cells in spotty belly are present either in the superficial layer or in the middle layer and they are relatively larger in dimension than the ones in Japanese bluefish.

Occasionally, in devil stinger and stone fish, between epithelial cells, multicellular glands which are supposed to granular glands are found and they occupied the 1/2 to 1/3 of thickness of the epidermis. However, they do not have histochemical reaction for mucosubstances as in the mucous cells.

The histochemical properties of mucosubstances of the skin in five teleostean species

The mucosubstances are found in the epidermis of all the five telesotean species studied. However, distribution property, and the amount of mucosubstances show differences in species and body regions. Results of the histochemical properties of mucosubstances in five teleostean species using conventional methods are outlined in Tables 1~5.

In spotty belly (Figs. 1A and 1B) and Japanese bluefish (Figs. 1K and 1L), most mucous cells in the all studied region stain weakly with PAS and are unreactive with AB at pH 2.5, thus their secretory products are likely to be neutral mucin.

In devil stinger (Figs. 1C and 1D), the mucous layer stains with both AB at pH 2.5 and PAS, thus they contain a mixture of neutral and acidic mucin. It shows bluish purple with AF pH 1.7-AB pH 2.5, thus the properties of acidic mucin are probably both sulfated and non-sulfated (sialyated) (Fig. 1E). The superficial cells of devil stinger contain mucosubstances, although the degree of staining is different in body region; in abdomen, they are positively reacted to both PAS and AB at pH 2.5 and stained bluish purple with AF at pH 1.7-AB at pH 2.5, thus their secretory products were likely to be a mixture of neutral, sulfated, and non-sulfated (sialyated) mucin, and in thorax and dorsum, they are positive to PAS but negative to AB at pH 2.5 (Figs. 1C~1E).

The mucous layer of stone fish is positive with AB at pH 2.5 and negative with PAS, thus it is acidic mucin (Fig. 1F). It shows bluish purple with AF at pH 1.7-AB at pH 2.5, property of acidic mucin is likely to be both sulfated and non-sulfated (sialyated) mucin (Fig. 1G). The superficial epithelial cells in abdominal and thoracic skin of stone fish are positive with PAS and negative with AB at pH 2.5, they contain only neutral mucin (Figs. 1F and 1G).

In cubed snailfish, the epithelial cells in the outmost give reaction for neutral and acidic mucopolysaccharides (Figs. 1H and 1I). They stain blue with AF pH 1.7-AB pH 2.5, thus, the property of acidic mucin is likely to be non-sulfated (sialyated) mucin (Fig. 1J).

The mucous cells of spotty belly and Japanese bluefish contain minimal amount of neutral mucin. In devil stinger and stone fish, the amount of mucin varies in body region. The mucous layer of devil stinger contains small amount of neutral mucin and moderate amount of acidic (sulfated and

Table 1. Staining properties with histochemistry of the epithelial elements in the epidermis of spotty belly, *Agrammus agrammus*

Regions	Epidermal elements	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5-PAS	AB pH 1.0-PAS	AF pH 1.7-AB pH 2.5
Abdominal skin	SEC	0	0	0	0	0	0
	MEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MG	±R	0	0	±R	±R	0
Thoracic skin	SEC	0	0	0	0	0	0
	MEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MG	±R	0	0	±>1R	±RP	0
Dorsal skin	SEC	0	0	0	0	0	0
	MEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MG	1±R	0	0	±R	±R	0

Degrees of staining: 4, very intense; 3, intense; 2, moderate; 1, weak; ±, trace; 0, absent; >, most marked
Abbreviations: R, red; RP, reddish purple; SEC, superficial epidermal cells; MEC, middle epidermal cells; BEC, basal epidermal cells; MC, mucous gland; AB, alcian blue; PAS, periodic acid Schiff; AF, aldehyde fuchsin

Table 2. Staining properties with histochemistry of the epithelial elements in the epidermis of devil stinger, *Inimicus japonicus*

Regions	Epidermal elements	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5-PAS	AB pH 1.0-PAS	AF pH 1.7-AB pH 2.5
Abdominal skin	ML	1R	2B	1B>2B	2BP	1~2BP	2BP
	SEC	1R	±B	0~±B	1RP,1BP	±~1RP	±BP
	MEC	0	0	0	0		0
	BEC	0	0	0	0		0
	MCG	0	0	0	0		0
Thoracic skin	ML	1R>±R	2B	1B>2B	2BP	1B	2BP
	SEC	1R>2R	0	0	1R	1R>±R	0
	MEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MCG	0	0	0	0	0	0
Dorsal skin	ML	1R>±R	2B	1B	2BP	1BP	2BP
	SEC	1R>±R	0	0	±R	±R	0
	MEC	0	0	0	0		0
	BEC	0	0	0	0		0
	MCG	0	0	0	0		0

Abbreviations: B, blue; BP, bluish purple; ML, mucous layer; MCG, multicellualr glands
Degrees of staining and other abbreviations are the same as Table 1.

Table 3. Staining properties with histochemistry of the epithelial elements in the epidermis of stone fish, *Erosa erosa*

Regions	Epidermal elements	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5-PAS	AB pH 1.0-PAS	AF pH 1.7-AB pH 2.5
Abdominal skin	ML	0	2B	±~1B	2B	1~2B	2BP
	SEC	±R	0	0	±~1R	±~1R	0
	MEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MCG	0	0	0	0	0	0
Thoracic skin	ML	0	2B>1B	1~2B	1~2B	1~2B	2BP
	SEC	±R	0	0	1~2R	2R	0
	MEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MCG	0	0	0	0	0	0
Dorsal skin	ML	0>±R	2B	2B	1~2B>1BP	1~2B>1BP	2BP>1BP
	SEC	0	0	0	0	0	0
	MEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MCG	0	0	0	0	0	0

Abbreviation: ML, mucous layer; MCG, multicellular glands
Degrees of staining and other abbreviations are the same as Table 1.

non-sulfated) mucin. The amount and property of mucins in superficial cells of devil stinger vary in body regions; in abdomen, the superficial epidermal cells have small amount of neutral mucin and minimal amount of acidic

Table 4. Staining properties with histochemistry of the epithelial elements in the epidermis of cubed snailfish, *Liparis tessellatus*

Regions	Epidermal elements	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5-PAS	AB pH 1.0-PAS	AF pH 1.7-AB pH 2.5
Abdominal skin	SEC	±R	±~1B	0	±~1BP, ±~1P	±R>±RP, ±BP	±~1B
	MEC	0	0>±B	0	0>±B	0	0>±B
	BEC	0	0	0	0	0	0
Thoracic skin	SEC	±R	±~1B	0	1B	0~±R	±~1B
	MEC	0	0>±~1B	0	0>±~1B	0	0>±B
	BEC	0	0	0	0	0	0
Dorsal skin	SEC	±R	±~1B	0	±~1B	±~1B	±~1B
	MEC	0	0>±B	0	0>±B	0	0>±B
	BEC	0	0	0	0	0	0

Abbreviation: P, purple
Degrees of staining and other abbreviations are the same as Table 1.

Table 5. Staining properties with histochemistry of the epithelial elements in the epidermis of Japanese bluefish, *Scombrops boops*

Regions	Epidermal elements	Stains					
		PAS	AB pH 2.5	AB pH 1.0	AB pH 2.5-PAS	AB pH 1.0-PAS	AF pH 1.7-AB pH 2.5
Abdominal skin	SEC	0	0	0	0	0	0
	IEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MC	±R	0	0	±R	±R	0
Thoracic skin	SEC	0	0	0	0	0	0
	IEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MC	±>1R	0	0	±>1R	±R	0
Dorsal skin	SEC	0	0	0	0	0	0
	IEC	0	0	0	0	0	0
	BEC	0	0	0	0	0	0
	MC	±R	0	0	±RP	±R	0

Degrees of staining and other abbreviations are the same as Table 1.

mucin, and in thoracic and dorsal skin, they contain small amount of neutral mucin. In stone fish, mucous layer have moderate amount of acidic mucin, and the superficial epidermal cells of abdominal and thoracic skin contain minimal amount of neutral mucin. In cubed snailfish, superficial epidermal cells contain minimal amount of neutral mucin and faint to small amount of non-sulfated (sialyated) mucin.

DISCUSSION

Within the epidermis of various vertebrates, there are two developmental pathways, one is related to keratinization and the other to mucogenesis. This duality in developmental potential is related to environmental adaptation; keratinizing cell types occur predominantly in terrestrial animals whereas mucus-producing cells are found in amphibious and aquatic forms.

The several types of unicellular glands, e.g., mucous cells, club cells, chloride cells, presented in teleost epidermis clearly separate it from the epidermis of other vertebrates. The most commonly encountered unicellular glands are the mucus-producing cells which have been studied extensively at the level of light microscopy (Mittal and Munshi, 1971; Mittal and Banerjee, 1974; Kazuyori and Motoyoshi, 1975; Mittal et al., 1980 and 1994; Singh and Mittal, 1990; Lee et al., 2000; Park and Kim, 2000 and 2007; Park et al., 2001 and 2005; Kim et al., 2002; Jeong and Jo, 2007). They are distributed more densely in regions of the body surface that are subjected to mechanical friction (Mittal and Munshi, 1971; Whitear and Mittal, 1984) or exposured to air (Mittal and Banerjee, 1974). A continuous secretion and shedding of mucus produced by mucous cells may prevent microbial colonization and infection, and various defence factors in the mucus, such as immunoglobulin, complement, lysozyme and lectin, may bestow protection (Harrell et al., 1976; Fletcher, 1982).

Conventional histochemical studies have shown that the mucous cells secreting mucus in epidermis of fish contained acidic and neutral mucopolysaccharides and the properties of mucosubstance varied in species. Fish inhabiting muddy waters and of burrowing habit have also been reported to secrete copious mucus (Mittal and Munshi, 1971; Mittal and Banerjee, 1974). Singh and Mittal (1990) suggested that in the two Indian major carp, *Catla catla* and *Cirrhina mrigala*, the mucous cells in intial stages of development appear to synthesize only neutral mucopolysaccharided, the acid mucopolysaccharides being

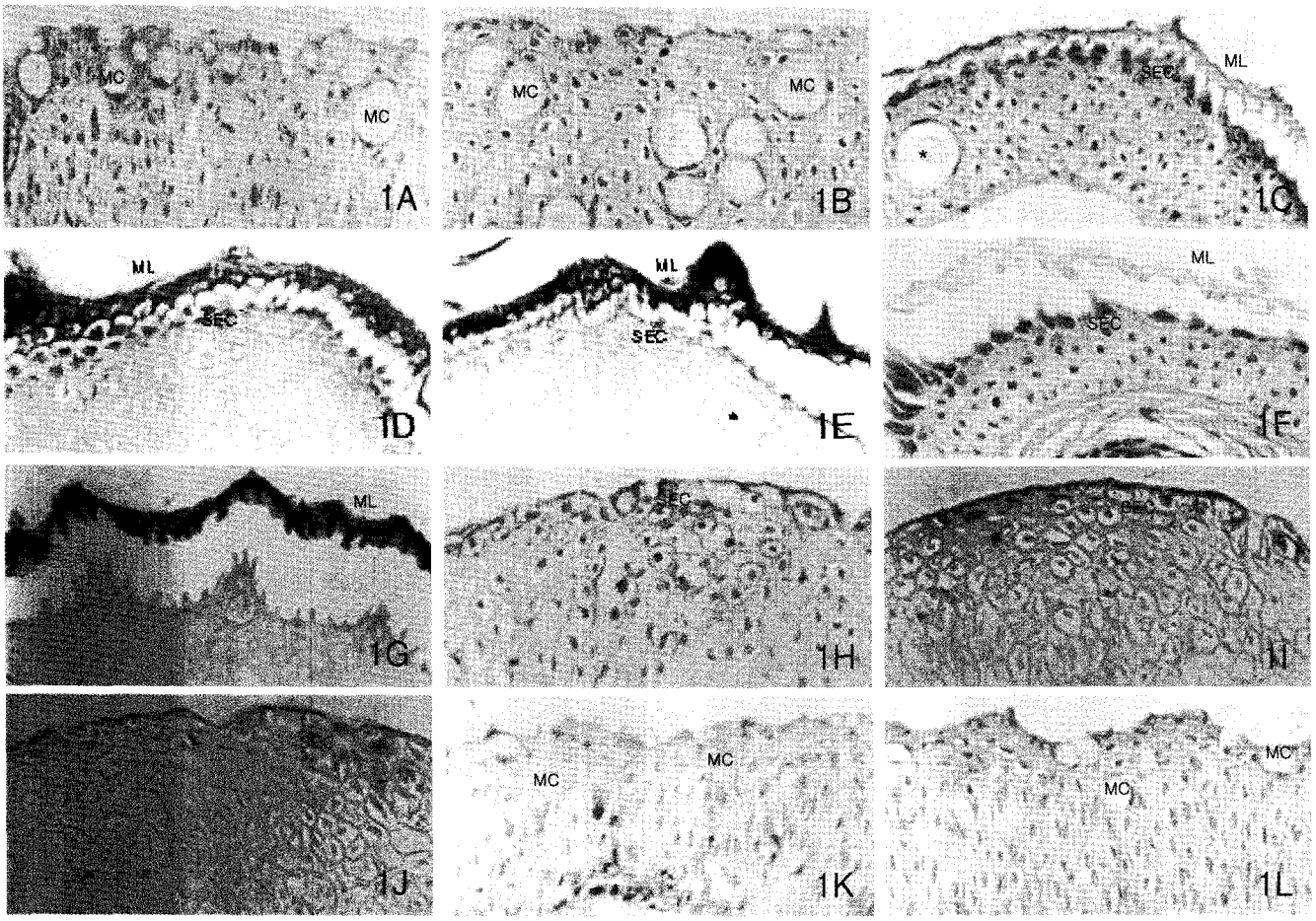

Fig. 1A. PAS staining in the abdominal skin of spotty belly, *Agrammus agrammus*. ×400. Most mucous cells (MC) give a faint red color.
Fig. 1B. AB at pH 2.5 staining in the abdominal skin of spotty belly, *Agrammus agrammus*. ×400. The mucous cells (MC) show no reaction.
Fig. 1C. PAS staining in the abdominal skin of devil stinger, *Inimicus japonicus*. ×400. The mucous layer (ML) and the superficial epidermal cells (SEC) give a weak red color. The multicellular glands (asterisk) are unreacive.
Fig. 1D. AB at pH 2.5-PAS staining in the abdominal skin of devil stinger, *Inimicus japonicus*. ×400. The mucous layer (ML) stains moderate bluish purple and the superficial epidermal cells (SEC) stain weak reddish purple or bluish purple.
Fig. 1E. AF at pH 1.7-AB at pH 2.5 staining in the abdominal skin of devil stinger, *Inimicus japonicus*. ×400. The mucous layer (ML) stains moderate bluish purple and the superficial epidermal cells (SEC) give a faint bluish purple color. The multicellualr gland (asterisk) is unreactive.
Fig. 1F. PAS staining in the abdominal skin of stone fish, *Erosa erosa*. ×400. The mucous layer (ML) is unreactive, whereas the superficial epidermal cells (SEC) stain faint red.
Fig. 1G. AF at pH 1.7-AB at pH 2.5 staining in the abdominal skin of stone fish, *Erosa erosa*. ×400. The mucous layer (ML) stain moderate bluish purple, whereas the superficial layer epithelial cells are unreactive.
Fig. 1H. PAS staining in the abdominal skin of cubed snailfish, *Liparis tessellatus*. ×400. The superficial epidermal cells (SEC) give a faint to weak red color.
Fig. 1I. AB at pH 2.5-PAS staining in the abdominal skin of cubed snailfish, *Liparis tessellatus*. ×400. The superficial epidermal cells (SEC) give a faint to weak bluish purple or purple color and a few middle layer epithelial cells give a faint blue color.
Fig. 1J. AF at pH 1.7-AB at pH 2.5 staining in the abdominal skin of cubed snailfish, *Liparis tessellatus*. ×400. The superficial epidermal cells (SEC) give a faint to weak blue color.
Fig. 1K. PAS staining in the thoracic skin of Japanese bluefish, *Scombrops boops*. ×400. The mucous cells (MC) stain faint red.
Fig. 1L. AB at pH 2.5 staining in the thoracic skin of Japanese bluefish, *Scombrops boops*. ×400. The mucous cells (MC) show no reaction.

gradually incorporated as they are further differentiated.

The epidermis of five teleostean species investigated in the present study as in most fishes, was mucogenic in nature. The unicellular mucous cells, the type commonly found in fishes were found in spotty belly and Japanese bluefish. However, they are completely lacking in the epidermis of devil stinger, stone fish, and cubed snailfish, although mucosubstances were observed on the skin surface of these species. The epidermal surface of devil stinger and stone fish was found to be covered with mucous layer. The superficial epidermal cells in devil stinger, stone fish, and cubed snailfish were mucus-secreting cells. Superficial epidermal cells as well as goblet mucous cells are known to secrete mucus to the body surface in *Blennius pholis* (Whitear and Mittal, 1984). Zhang et al. (2003) reported that the mucous cells, the type commonly found in

fishes, were not found in the epidermis of *Periophthalmus* species, although mucus-like fuzzy materials were sometimes found on the skin surface of these species, and suggested that these species have some unknown mechanism for producing mucus. I suggest that the observed mucous layers of devil stinger and stone fish to protect the integument from abrasion and desiccation come from the secretory products of epithelial cells.

In the present study, the mucous cells of spotty belly and Japanese bluefish contain only neutral mucin. This is the same as the property of mucosubstance in blenny, *Pholis nebulosa* (Lee et al., 2000a) and bastard halibut, *Paralichthys olivaceus (*Kim et al, 2002). The epidermal mucosubstance of devil stinger and stone fish is a mixture of neutral and acidic (sulfated and non-sulfated) mucin. In cubed snailfish, the type of epithelial mucosubstances is identified as a mixture of neutral and acidic (non-sulfated) mucin. These are the same as the property of mucosubstance in the common carp and three Indian major carps (Singh and Mittal, 1990).

The skin is a complex epithelium and the most extensive interface between the animals and its external environment. In the present study, differences of skin structure and qualitative differences in the chemical composition of the epidermal mucus with interspecies of fish have been observed. Such differences may reflect an adaptation to the vastly different habitats of teleost fish and more comparative studies are needed.

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