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The Pharyngeal Organ in the Buccal Cavity of the Male Siamese Fighting Fish, *Betta splendens*, Supplies Mucus for Building Bubble Nests

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The male Siamese fighting fish, *Betta splendens*, builds a bubble nest on the water surface to care for offspring during the reproductive period. To our knowledge, this study is the first to determine the composition of the bubble nest and to compare the pharyngeal organs of male and female Siamese fighting fish to determine the relationship between the pharyngeal organ and the ability to make bubble nests. Dot blots of the bubble nest probed with periodic acid-Schiff's (PAS) staining and Ponceau S solution revealed that the contents of the nest are glycoprotein rich. Dissection of the heads of Siamese fighting fish showed that the pharyngeal organ is located in the position through which inhaled air passes. The epithelial structure of the pharyngeal organ of the Siamese fighting fish, like that of other teleosts, has numerous wrinkles and papillae. Mucous goblet cells were observed on the epithelium of pharyngeal organs in male and female fish. The pharyngeal organ was found to be larger in male than in female fish. In addition, the epithelium of the pharyngeal organ in male fish has a greater number of mucous goblet cells than that in female fish. In Siamese fighting fish, this sexual dimorphism of the pharyngeal organ suggests that the male fish secretes more glycoprotein-rich mucus to build the bubble nest. Future work will focus on the type of mucous cells found in the epithelium of the pharyngeal organ that contributes to bubble formation and will determine the components of the mucus in the bubble nest.

Key words: pharyngeal organ, bubble nest, Siamese fighting fish, mucus, sexual dimorphism

INTRODUCTION

The pharyngeal organs of teleosts are a pair of bean-shaped structures that have numerous papillae and wrinkles on the surface. They lie antero-dorsal to and are diverticula of the posterior region of the roof of the pharynx (Miller, 1964; Sanderson et al., 1991; Bauchot et al., 1993). The epithelia of pharyngeal organs are rich in mucous cells, which produce more mucus to retain the particle-mucus aggregates of the feeding apparatus in teleosts (Miller, 1964; Sibbing et al., 1986; Sibbing, 1988; Sanderson et al., 1991, 1998; Yashal et al., 2007). In the epithelium of teleosts, mucous-cell types include goblet cells, secretory cells, and non-secretory cells. The major constituents of mucus are glycoproteins (Shephard, 1994). Previous studies have revealed that periodic acid-Schiff's (PAS) staining can label all types of mucous cells in the epithelium of teleosts (Gona, 1979; Yashal et al., 2007; Mittal and Mittal, 2008).

The Siamese fighting fish, *Betta splendens*, is an anabantoid, or labyrinth, fish. These fish can directly exchange gas from the air on the water surface through air-breathing organs. The air-breathing organ of the Siamese fighting fish is a pair of labyrinths covered with a respiratory epithelium in the suprabranchial chambers (Graham, 1997). The anabantoid fishes include 6 genera and 127 species. Diverse reproductive behaviors are found in these species, including free spawners that do not exhibit parental care, substrate spawners exhibiting male parental care, bubble nesters exhibting male or biparental care, and male or, rarely, female mouthbrooders (Rüber, 2004). The Siamese fighting fish is a bubble nester exhibiting male care (Bronstein, 1982). The male fish gulps air into the buccal cavity from the water surface, and then exhales bubbles below the water surface to make a mucus-coated floating bubble nest during the reproductive period (Shephard, 1994). In the region of this bubble nest, a male fish courts the female fish and defends a territory from other fish in the area. After breeding, the male fish cares for the fertilized eggs in the bubble nest until the embryos have developed into larvae (Bronstein, 1982). The functions of bubble nests are to protect the embryos and to supply more oxygen or nutrition to the embryos. The size of the bubble nest built by a male fish affects the reproductive choices of female fish and the survival rate of larvae (Jaroensutasinee and Jaroensutasinee, 2001a, b).

Because of these behavioral characteristics of the male Siamese fighting fish, this species has proven to be a good model animal for studying the relationship between aggressive behavior and reproduction (Bronstein, 1982; Craft et al., 2003; Jaroensutasinee and Jaroensutasinee, 2003; Matos

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et al., 2003). However, studies of the constituents of the bubble nest in this species are limited. The functions of the pharyngeal organs in bubble-nest-making anabantoid fish have also not been examined previously. The present study focuses on the relationship between the pharyngeal organ and the bubble nest in Siamese fighting fish. We used dot blots on PVDF membranes with PAS staining and Ponceau S solution to verify the constituents of the bubble nest made by male Siamese fighting fish, and observed the location and ultrastructure of pharyngeal organs. Moreover, we compared the sizes of the pharyngeal organs in male and female fish. Finally, we quantified the numbers of mucous cells on the epithelia of the pharyngeal organs in male and female fish to determine their differential abilities to secrete mucus.

MATERIALS AND METHODS

Fish and experimental environments

Mature male and female Siamese fighting fish (*Betta splendens*; 3.0 ± 0.5 cm), a bubble-nesting anabantoid teleost species, were purchased from a local aquarium and kept in the laboratory for at least two weeks at 28 ± 1°C under photoperiod 12L:12D. Each sampling system consisted of one male and one female fish kept in the same tank without air pumping. A transparent container was used to separate the female from the male to protect the female fish from attack by the male and to stimulate the male to make a bubble nest in the sampling system. Fish were fed a daily diet of commercial pellets. The water in each tank was partially refreshed every week.

Collection and dot blotting of bubble nests

The bubble nest on the water surface made by each male Siamese fighting fish was collected in a tube using a dropper. Tap water from the same tank was also collected as a control. The samples were centrifuged at 4°C and 1000 × g for 10 min. The supernatants were loaded onto PVDF membranes (0.45 μm^2, Millipore, Billerica, MA, USA) that were hydrated with methanol and 0.1 M PB. Each dot-blotted membrane was oxidized in 0.5% aqueous periodic acid for 10 min, and then rinsed in distilled water. The membrane was then treated with Schiff's reagent (Merck, Darmstadt, Germany) for 15 min to stain the glycoprotein, followed by washing in running tap water for 10 min. In addition, the dot-blotted membrane was treated with 0.1% Ponceau S solution to probe the protein content (Salinovich and Montelaro, 1986).

Location of the pharyngeal organ

Five Siamese fighting fish were fixed in formalin for two days (after anesthetization with MS222, 100–200 mg/L). The fixed fish were washed three times with 0.1 M phosphate buffer (PB, pH 7.2) for 10 min. To reveal the location of the pharyngeal organ in the buccal cavity, the head of each fish was longitudinally sectioned and cross-sectioned by autopsy knife.

Ultrastructure of the pharyngeal organ

The morphology of the pharyngeal organ and the openings of mucous cells in Siamese fighting fish were examined by scanning electron microscopy. Pharyngeal organs were excised from the buccal cavity (after anesthetization with MS222, 100–200 mg/L) and fixed at 4°C in a fixative consisting of 5% (v/v) glutaraldehyde and 4% (w/v) paraformaldehyde in 0.1 M PB for 12 hr. Fixed pharyngeal organs were rinsed for 15 min with three changes of 0.1 M PB at 4°C and then post-fixed with 1% (w/v) osmium tetroxide in 0.1 M PB for 1.5 hr. After post-fixation, the pharyngeal organs were rinsed in PB and dehydrated in ascending concentrations of ethanol from 30% to absolute. Samples were then critical-point dried using liquid CO_2 in a Hitachi HCP-2 (Tokyo, Japan) critical-point drier, mounted on aluminum stubs with silver paint, and sputter coated for 3 min with a gold-palladium complex in a Pt Coater. The coated specimens were observed using a scanning electron microscope (JEOL JSM-6700F, Tokyo, Japan).

Size of the pharyngeal organ

Five pharyngeal organs were excised from the buccal cavities of (a) male Siamese fighting fish (*B. splendens*) with bubble nests, (b) male fish without bubble nests, and (c) female fish (which cannot make nests) after anesthetization with MS222 (100–200 mg/L). The organs were wiped with tissue paper to remove water and blood. The mass (mg) of each pharyngeal organ and the standard body length (cm) of each fish were measured. The pharyngeal organs were then fixed in Bouin's solution (Sigma, Saint-Quentin-Fallavier, France) for 48 hr.

Paraffin section and staining

After fixation with Bouin's solution, pharyngeal organs were dehydrated through a graded ethanol series, infiltrated with xylene, and embedded in paraffin. Serial 7-μm sections were mounted on gelatin-coated glass slides. The sections were oxidized in 0.5% aqueous periodic acid for 10 min and then rinsed in distilled water. The slides were then treated with Schiff's reagent (Merck, Darmstadt, Germany) for 15 min, followed by washing in running tap water for 10 min and counterstaining with hematoxyline. The prepared sections were observed under a light microscope (Olympus BX-50, Tokyo, Japan).

Quantification of the number of PAS-positive cells

Most PAS-positive cells were distributed in the epithelium of the

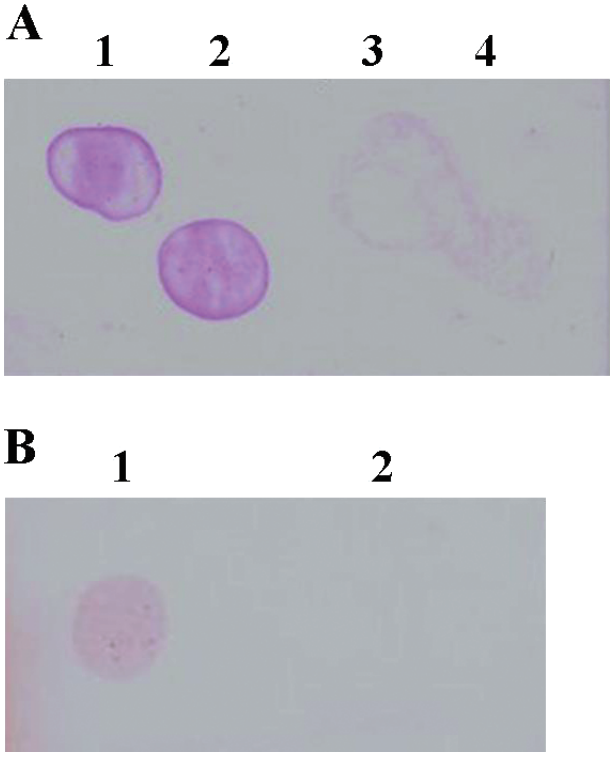

Fig. 1. Supernatants from bubble nests made by male Siamese fighting fish (*B. splendens*) dot-blotted on a PVDF membrane and stained with PAS **(A)** and Ponceau S **(B)**. Magenta blots (A1, 5 μl; A2, 10 μl) indicate PAS-positive signals on the membrane. The red blot is a protein-positive signal stained with Ponceau S (B1). Tap water to which fish had become acclimated was used as the loading control (A3, 5 μl; A4, 10 μl; B2) (n = 3).

papillae in the pharyngeal organs of all observed Siamese fighting fish. For each sample, 15 areas on the epithelium of the papillae were randomly selected. The length of each epithelial region was measured to standardize cell counts to a fixed length (100 μm).

Statistical analysis

Values were compared using an unpaired one-way analysis of variance (ANOVA) (Tukey's pair-wise method), and $P < 0.05$ was set as the level of significance. Values are expressed as means ± S.E.M. (the standard error of the mean) unless stated otherwise.

RESULTS

PAS-stained PVDF membranes dot-blotted with (i) bubble nests collected from the water surface and (ii) tap water from the same tank with each bubble nest showed that the blots of bubble nests exhibited stronger PAS-positive reactions, while the PAS reactions of the blots of tap water were weaker (Fig. 1A). When these membranes were stained with Ponceau S solution, a protein signal appeared on the dot-blots of bubble nests, while no signal was found on the blots of tap water (Fig. 1B).

The pharyngeal organs of a mature Siamese fighting fish are a pair of bean-shaped structures (Figs. 2B; 3A). They lie antero-dorsal to and are diverticula of the posterior region of the roof of the pharynx (Fig. 2A; 2B), through which exhaled air from the labyrinth passes. The esophagus opens caudally from the expanded roof of the pharynx (Fig. 2A; 2B). Scanning electron micrographs revealed that the pharyngeal organs of both male and female fish possess many wrinkles, papillae, and pharyngeal teeth; these teeth are circled by papillae (Fig. 3). On the surface of the papillae,

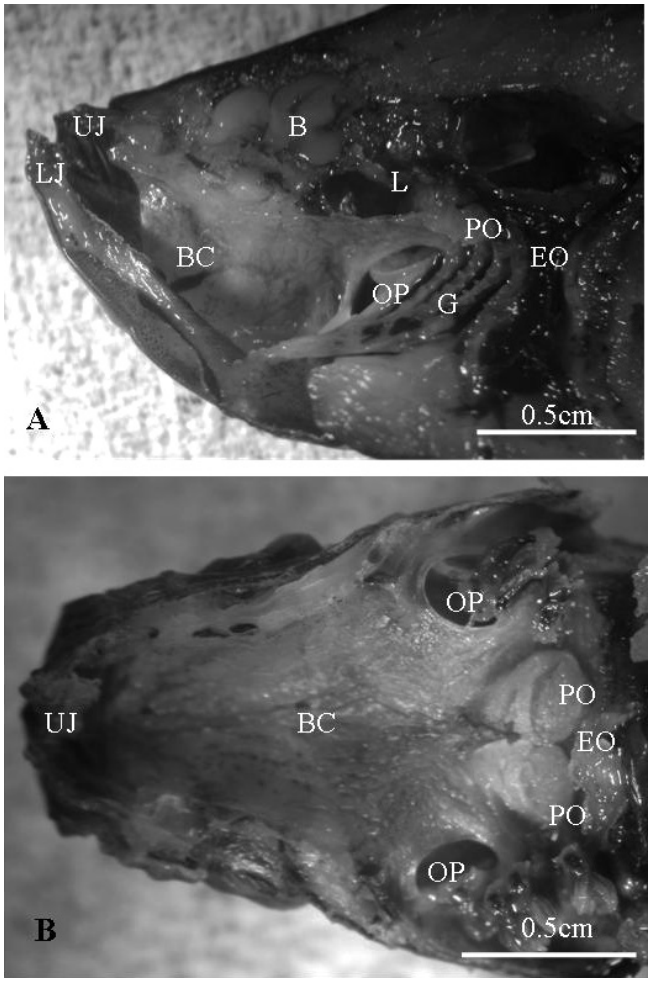

Fig. 2. External and internal morphology of the oral cavity of Siamese fighting fish (*B. splendens*), showing the locations of the pharyngeal organs (PO), through which exhaled air passes. **(A)** Sagittal section of the head; **(B)** head-on view of the upper jaw. B: brain; BC: buccal cavity; E: eye; EO: esophagus opening; G: gill; L: labyrinth; LJ: lower jaw; O: operculum; OP: pharyngeal opening; UJ: upper jaw.

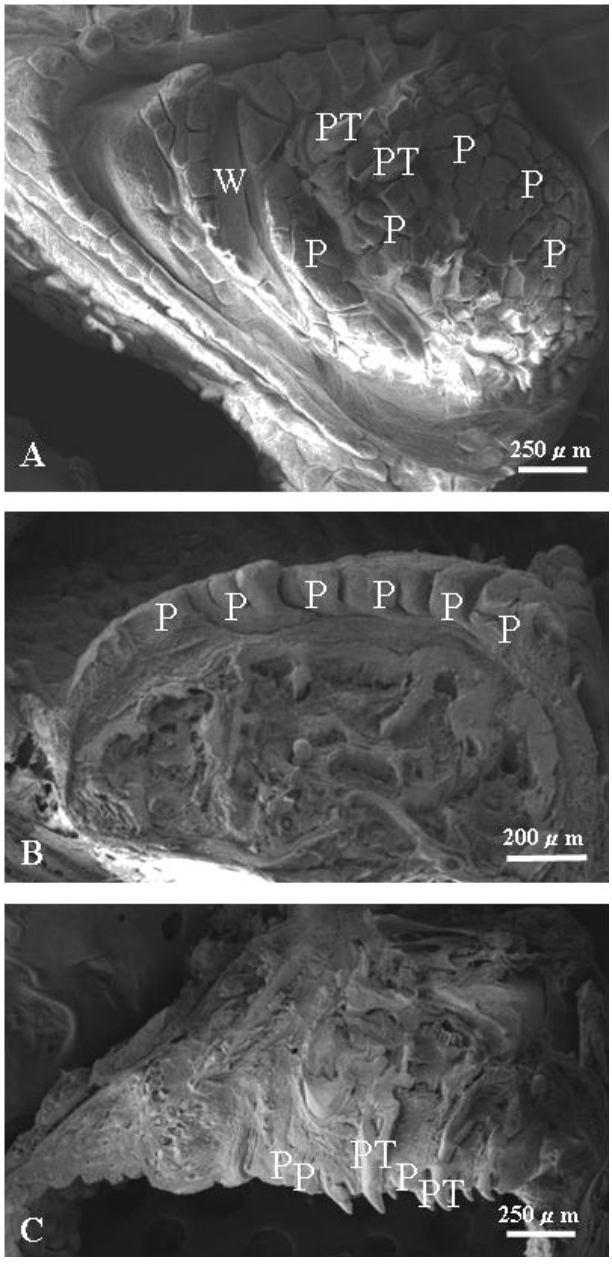

Fig. 3. Scanning electron micrographs of the pharyngeal organs of Siamese fighting fish (*B. splendens*). Numerous papillae (P) and wrinkles (W) are present on the pharyngeal organ. **(A)** External structure; **(B)** horizontal section; **(C)** longitudinal section. PT: pharyngeal teeth. (× 50).

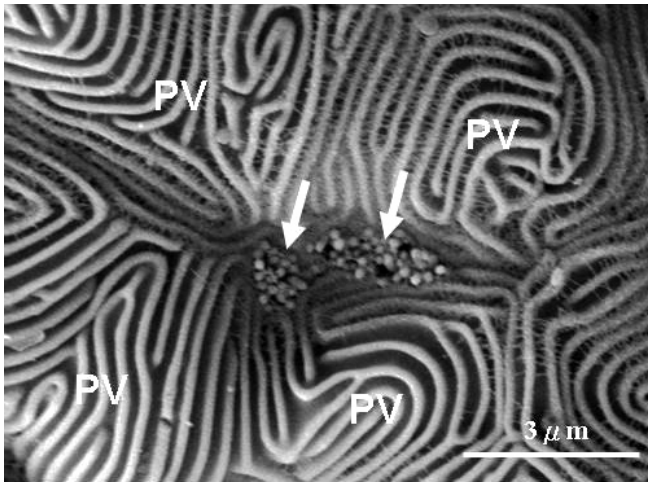

Fig. 4. The mucous-cell-like opening (arrow) on the papillae of the pharyngeal organ in a Siamese fighting fish (*B. splendens*) observed by scanning electron microscopy (× 10000). Mucus granules are present in the mucous-cells-like opening. PV: pavement cells.

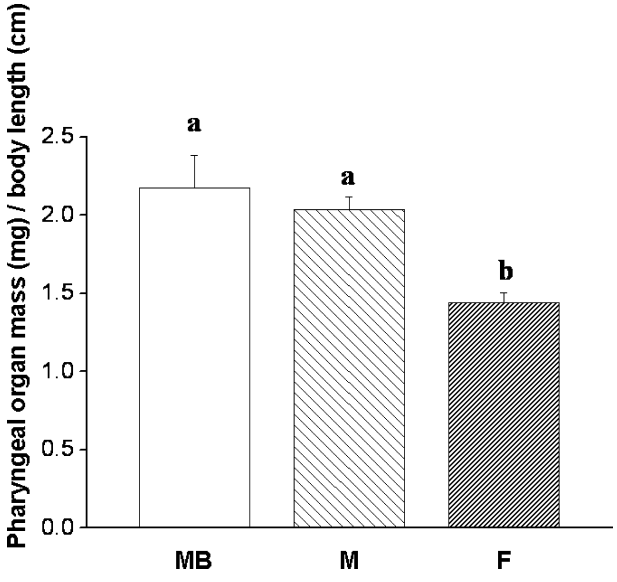

Fig. 5. Pharyngeal-organ mass (mg) and body length (cm) in male Siamese fighting fish (*B. splendens*) with bubble nests (MB, white panel), male fish without bubble nests (M, sparsely hatched panel), and female fish (F, densely hatched panel). As indicated by dissimilar letters, the pharyngeal organ was significantly smaller in female fish than in the other groups (n = 5, one-way ANOVA followed by Tukey's comparison, $P < 0.05$). Values are represented as the means ± S.E.M.

many mucous-cells-like openings with mucus granules were observed (Fig. 4). The average size of the pharyngeal organs was found to be sex-dependent. Female Siamese fighting fish had smaller pharyngeal organs. The average size in all male fish was about 1.5-fold greater than that in female fish. There was no significant difference in pharyngeal-organ size between male fish with and without bubble nests (Fig. 5).

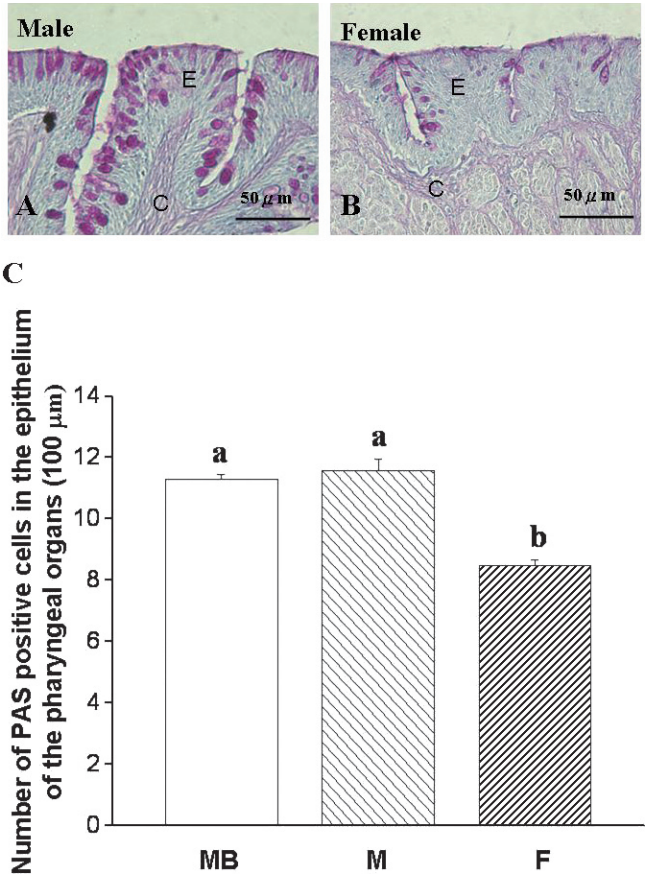

Fig. 6. PAS-positive cells (magenta) found on the epithelia of pharyngeal organs in **(A)** male and **(B)** female Siamese fighting fish (*B. splendens*) by PAS staining. **(C)** Quantification of PAS-positive cells in the pharyngeal organs of male Siamese fighting fish with bubble nests (MB, white panel), male fish without bubble nests (M, sparsely hatched panel), and female fish (F, densely hatched panel). As indicated by dissimilar letters, there were significantly fewer PAS-positive cells in the pharyngeal organs of female fish than in those of either group of male fish (n = 5, one-way ANOVA followed by Tukey's comparison, $P < 0.05$). Values are represented as means ± S.E.M. C: connective tissue; E: epithelium.

Longitudinal sections of the pharyngeal organs of Siamese fighting fish stained with periodic acid-Schiff's (PAS) and hematoxyline revealed numerous PAS-positive cells in the epithelial region of the papillae (Fig. 6A, B). Quantification of the PAS-positive cells in the epithelium of the papillae indicated that the number of cells in male fish was greater (approximately 1.4-fold) than that in female fish, but no significant difference was found between male fish with and without bubble nests (Fig. 6C).

DISCUSSION

Building bubble nests on the water surface, a specialized behavior during the reproductive period, is observed in anabantoid fish (Bronstein, 1982; Rüber, 2004), snakehead fish (Ahmad and Hasnain, 2005), catfish (Andrade and Abe, 1997), and frogs (Copper et al., 2005). Shephard (1994) has reported that the mucus-coated bubbles of the nest are formed from air and mucus in the buccal cavity of the Siamese fighting fish. To our knowledge, the present study

is the first to reveal that the bubble nests of male Siamese fighting fish are made up of glycoprotein-rich mucus (Fig. 1). The mucus is highly viscous, enabling the bubbles to persist for longer periods in the water. The male fish can aggregate the bubbles to nest. Previous studies have indicated that glycoproteins reduce bacterial and fungal infections in fish (Shephard, 1994). In addition, Bronstein (1982) has reported that the bubble nests built by male Siamese fighting fish improve the hatching rate of the embryos, suggesting that the mucus-coated bubbles reduce bacterial and fungal infections in the embryos of Siamese fighting fish.

In Siamese fighting fish, the pharyngeal organ is located on the dorsal pharynx where the four pairs of gills converge. This air-breathing fish species has a pair of labyrinths in the suprabranchial chambers whose pharyngeal opening is located adjacent to the pharyngeal organ (Fig. 2A; B). Graham (1997) has reported that anabantoid fish inhale air from the water surface to the buccal cavity and that the air then passes across the pharyngeal opening into the labyrinth. Following gas exchange, the air is exhaled through the same opening to the mouth or released from the gill slits. Hence, based on the position of the pharyngeal organ, our study suggests that the air used to make bubble nests is gulped from the water surface and then contacts the pharyngeal organ and is mixed with mucus in the buccal cavity of the Siamese fighting fish.

Numerous teeth, wrinkles, and papillae are present in the pharyngeal organ of the Siamese fighting fish (Fig. 3). The structure of the pharyngeal organ is similar to that of other teleosts (Miller, 1964; Sanderson et al., 1991; Bauchot et al., 1993). The teeth of the pharyngeal organ may masticate and retain food in the buccal cavity of Siamese fighting fish. Previous studies have indicated that the wrinkles and papillae of the pharyngeal organ expand the mucus-secreting epithelial surface (Miller, 1964; Sanderson et al., 1991; Bauchot et al., 1993). In agreement with previous studies focused on the ultrastructural crypts of mucous cells (Marshall et al., 1997), our scanning electron micrographs show mucous-cell-like openings on the surface of the papillae in Siamese fighting fish (Fig. 4).

Our results also demonstrate that the average size of the pharyngeal organs of male Siamese fighting fish is greater than that of female fish (Fig. 5), although the structure of the pharyngeal organs of male and female fish is identical (data not shown). A larger pharyngeal organ may have a larger epithelial surface. In addition, PAS-positive cells occur in the epithelia of wrinkles in the pharyngeal organs (Fig. 6A; B). Previous studies have reported that mucous goblet cells in the epithelium produce stronger PAS signals in teleosts (Gona, 1979; Yahpal et al., 2007). Together with the histological characteristics of goblet cells, we have compared these numbers of PAS-positive cells in the epithelium of the pharyngeal organs between the male and female Siamese fighting fish (Fig. 6C). The larger epithelial surface and greater number of mucous goblet cells in the pharyngeal organs of male fish indicate a greater capacity for mucus secretion than in those of female fish. Because mucous goblet cells are also present in the pharyngeal organs of female fighting fish, mucus secretion from the pharyngeal organs of male and female fish may be multifunctional, perhaps used both in feeding and in building bubble nests. Furthermore, Siamese fighting fish may secrete more mucus to moisten the oral and suprabranchial cavities because they breathe air, just as terrestrial animals produce more mucus in the respiratory system (Jackson, 2001). Our comparison of the size of the pharyngeal organ and the number of mucous cells reveals no significant difference between male fish with and without bubble nests (Figs. 5; 6). These results indicate that the sexual dimorphism in mucus secretion in the pharyngeal organs of mature male fish is not limited to the reproductive period. Previous studies have reported that the mucus secretion of goblet cells in mammals is under hormonal and neuronal controls (Shimura, 2000). Schwagmeyer et al. (1977) observed that the ability of the anabantoid species *Macropodus opercularis* to build bubble nests decreases after telencephalon ablation. Therefore, the mucus-secreting capacity of the pharyngeal organs of male Siamese fighting fish for building bubble nests is probably under hormonal and neuronal controls during the reproductive period.

Sexual dimorphism has been found in the reproductive mechanisms of many fish, including anabantoid species (Cambray, 1997; Jaroensutasinee and Jaroensutasinee, 2001b). In teleosts, this dimorphism increases the reproductive advantage of male or female fish by resulting in the expression of specific reproductive behaviors (Parker, 1992). The present study indicates that the pharyngeal organs of Siamese fighting fish are sexually dimorphic. Because the mucus-secretion capacities of the pharyngeal organs are sex dependent, our results suggest that sexual dimorphism in the pharyngeal organs of male Siamese fighting fish supplies greater mucus secretion in the buccal cavity for building the bubble nest.

Taken together, our observations provide evidence that the bubble nests built by male Siamese fighting fish are composed of glycoproteins and that the pharyngeal organs of this species exhibit sexual dimorphism for mucus secretion. To illustrate the advantages of building bubble nests for breeding offspring, future studies will investigate the type of mucous cells in the epithelium of the pharyngeal organs that is used to form bubbles, and will seek to determine the components of the mucus in bubble nests.

REFERENCES

Ahmad R, Hasnain A (2005) Ontogenetic changes and developmental adjustments in lactate dehydrogenase isozymes of an obligate air-breathing fish *Channa punctatus* during deprivation of air access. Comp Biochem Physiol B 140: 271–278

Andrade DV, Abe AS (1997) Foam nest production in the armoured catfish. J Fish Biol 50: 665–667

Bauchot R, Ridet JM, Diagne M (1993) The epibranchial organ, its innervation and its probable functioning in *Heterotis niloticus* (Pisces, Teleostei, Osteoglossidae). Env Biol Fish 37: 307–315

Britz R, Cambray JA (2001) Structure of egg surfaces and attachment organs in anabantoids. Ichthyol Explor Freshwaters 12: 267–288

Bronstein PM (1982) Breeding, paternal behavior, and their interruption in *Betta splendens*. Anim Learn Behav 10: 145–151

Cambray JA (1990) Early ontogeny and notes on breeding behaviour, habitat preference and conservation of the Cape kurper, *Sandelia capensis*. Ann Cape Prov Mus Nat Hist 18: 159–182

Cambray JA (1997) The spawning behaviour of the endangered Eastern Cape rocky *Sandelia bainsii* (Anabantidae), in South

Africa. Environ Biol Fish 49: 293–306
Cooper A, Kennedy MW, Fleming RI, Wilson EH, Videler H, Wokosin DL, Su TJ, Green RJ, Lu JR (2005) Adsorption of frog foam nest proteins at the air–water interface. Biophys J 88: 2114–2125
Craft BB, Velkey AJ, Szalda-Petree A (2003) Instrumental conditioning of choice behavior in male Siamese fighting fish (*Betta splendens*). Behav Processes 63: 171–175
Fletcher TC, Jones R, Reid L (1976) Identification of glycoproteins in goblet cells of epidermis and gills of plaice (*Pleuronectes platessa* L.), flounder (*Platichthys flesus* (L.)) and rainbow trout (*Salmo gairdneri* Richardson). Histochem J 8: 597–608
Gona G (1979) Mucous glycoproteins of teleostean fish: a comparative histochemical study. Histochem J 11: 709–718
Graham JB (1997) Air-breathing fishes: evolution, diversity, and adaptation. Acdemic Press, San Diego, CA
Jackson AD (2001) Airway goblet-cell mucus secretion. Trends Pharmacol Sci 22: 39–45
Jaroensutasinee M, Jaroensutasinee K (2001a) Bubble nest habitat characteristics of wild Siamese fighting fish. J Fish Biol 58: 1311–1319
Jaroensutasinee M, Jaroensutasinee K (2001b) Sexual size dimorphism and male contest in wild Siamese fighting fish. J Fish Biol 59: 1614–1621
Jaroensutasinee M, Jaroensutasinee K (2003) Type of intruder and reproductive phase influence male territorial defence in wild-caught Siamese fighting fish. Behav Processes 64: 23–29
Marshall WS, Bryson SE, Darling P, Whitten C, Patrick M, Wilkie M, Wood CM, Buckland-Nicks J (1997) NaCl transport and ultrastructure of opercular epithelium from a freshwater–adapted euryhaline teleost, *Fundulus heteroclitus*. J Experiment Zool 277: 23–37
Matos RJ, Peake TM, McGregor PK (2003) Timing of presentation of an audience: aggressive priming and audience effects in male displays of Siamese fighting fish (*Betta splendens*). Behav Processes 63: 53–61
Miller RV (1964) The morphology and function of the pharyngeal organs in the clupeid, *Dorosoma petenense* (Gunther). Chesapeake Sci 5: 194–199
Mittal SP, Mittal AK (2008) Glycoproteins in the epithelium of lips and associated structures of a hill stream fish *Garra lamta* (*Cyprinidae, Cypriniformes*): a histochemical investigation. Anat Histol Embryol 37: 101–113
Parker GA (1992) The evolution of sexual size dimorphism in fish. J Fish Biol 41: 1–20
Peters HM (1978) On the mechanism of air ventilation in anabantoids (Pisces: Teleostei). Zoomorphology 89: 93–123
Pickering AD, Macey DJ (1977) Structure histochemistry and the effect of handling stress on the mucous cells of the epidermis of the char *Salvelinus alpinus* (L.). J Fish Biol 10: 505–512
Rüber L, Britz R, Tan HH, Ng PK, Zardoya R (2004) Evolution of mouthbrooding and life–history correlates in the fighting fish genus *Betta*. Evolution 58: 799–813
Salinovich O, Montelaro RC (1986) Reversible staining and peptide mapping of proteins transferred to nitrocellulose after separation by sodium dodecylsulfate–polyacrylamide gel electrophoresis. Anal Biochem 156: 341–347
Sanderson SL, Cech JJ Jr, Patterson MR (1991) Fluid dynamics in suspension-feeding blackfish. Science 251: 1346–1348
Sanderson SL, Mort ME, Cech JJ Jr (1998) Particle retention by non-suspension-feeding cyprinid fishes. Can J Fish Aquat Sci 55: 861–868
Schwagmeyer P, Davis RE, Kassel J (1977) Telencephalic lesions and behavior in the teleost *Macropodus opercularis* (L): effects of telencephalon and olfactory bulb ablation on spawning and foamnest building. Behav Biol 20: 463–470
Shephard KL (1994) Functions for fish mucus. Biol Fish 4: 473–485
Shimura S (2000) Signal transduction of mucous secretion by bronchial gland cells. Cell Signal 12: 271–277
Sibbing FA (1988) Specializations and limitations in the utilization of food resources by the carp, *Cyprinus carpio*: a study of oral food processing. Environ Biol Fish 22: 161–178
Sibbing FA, Osse JWM, Terlouw A (1986) Food handling in the carp (*Cyprinus carpio*): its movement patterns, mechanisms and limitations. J Zool 210: 161–203
Whitear M (1970) The skin surface of bony fishes. J Zool 160: 437–454
Yashpal M, Kumari U, Mittal S, Mittal AK (2007) Histochemical characterization of glycoproteins in the buccal epithelium of the catfish, *Rita rita*. Acta Histochem 109: 285–303

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