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Gross anatomy and histology of the hook and skin of forehead brooding male nurseryfish, *Kurtus gulliveri*, from northern Australia

Tim M. Berra[a] & John D. Humphrey[b]
[a]*Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Mansfield, OH 44906, U.S.A. (e-mail: berra.1@osu.edu)*
[b]*Department of Business, Industry and Resource Development, GPO Box 990, Darwin, NT 0801, Australia (e-mail: john.humphrey@nt.gov.au)*

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Synopsis

Mature males of nurseryfish have a hook on their head to which the eggs become attached and are carried like a bunch of grapes. This paper examines the anatomy and histology of the hook. The osteological basis of the hook is shown to be a modification of the supraoccipital crest of the skull covered by typical teleost skin. The integument in the cleft of the hook, where the eggs are attached, is considerably different from ordinary fish skin. The stratified epidermis is devoid of secretory mucus and neurosensory cells and is folded into crypts that extend deeply into the dermis. This may be a specialization that facilitates adhesion of the sticky egg mass. Field observations show that this cleft area of the hook is edematous, and histology confirms that the area is highly vascularized. We speculate that this may facilitate gas exchange and/or nutrition between the male and the egg mass, but this can only be confirmed by physiological experiments with 'pregnant males' in captivity. Engorgement with blood in the highly vascularised dermis of the hook may help hold the egg mass in place.

Introduction

The nurseryfish, *Kurtus gulliveri,* is an unusual fresh and brackish-water fish found in coastal waters of northern Australia and southern New Guinea (Berra 2001). It and its congener, *K. indicus*, which ranges from India to Borneo, constitute the perciform family Kurtidae which is placed in its own suborder, Kurtoidei (Nelson 1994). Very little is known of the biology of nurseryfish, and the few previously published papers on nurseryfish biology are about 90 years old. To remedy this situation, a study of *K. gulliveri* was begun on the Adelaide River, 50 km east of Darwin, Northern Territory, Australia from April–November 2001. The results reported here are part of that study. Other papers resulting from this study include a description of alimentary canal anatomy and diet which is composed of arthropods and small fishes (Berra & Wedd 2001). Spawning is believed to coincide with the dry season in Australia's Top End (May–November), based on the findings of egg masses and nurseryfish larvae in the Adelaide River during that period (Berra & Neira unpublished).

The most unusual feature of *K. gulliveri* is the presence of a hook on the head of mature males (Figure 1). Young males show only a slight protuberance that eventually enlarges as the fish grows. Females lack the hook. Somehow the eggs of this species become attached to the hook, and the male carries them around like a bunch of grapes (Figure 2). Normal teleost integument is refractory to the attachment of external objects and attachments of foreign objects are a feature of pathological states.

The highly unusual parental care system of nurseryfish was reported and illustrated by Weber (1910, 1913) and has been termed 'forehead brooding' (Balon 1975). Beaufort (1914) depicted the nurseryfish skeleton including the hook, which is formed from the supraoccipital crest of the skull (Figure 3). The skin covering

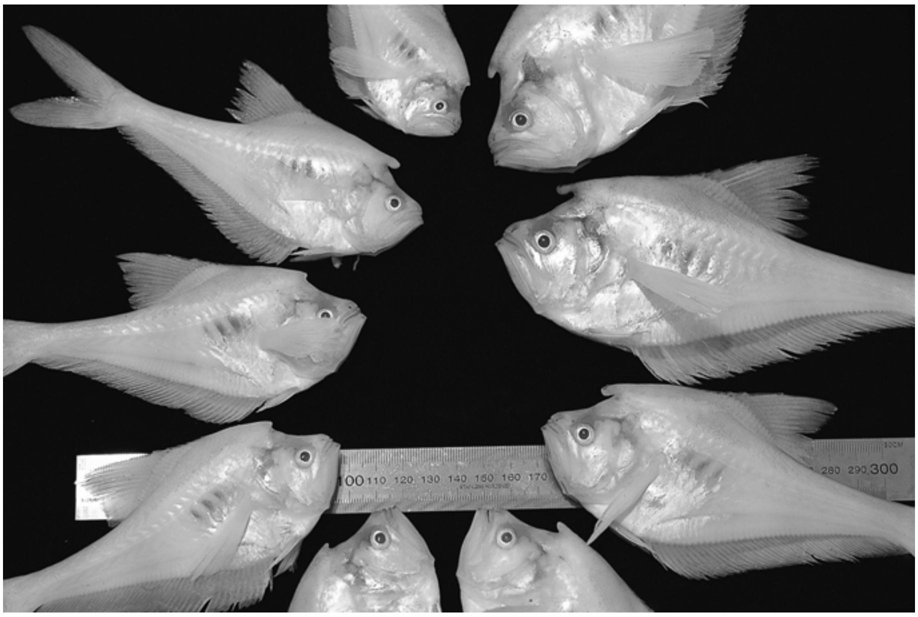


Figure 1. Male *Kurtus gulliveri* from a single collection on 11 September 2001 from Marrakai Creek showing different degrees of hook development. Size ranged from 106–185 mm standard length (SL).

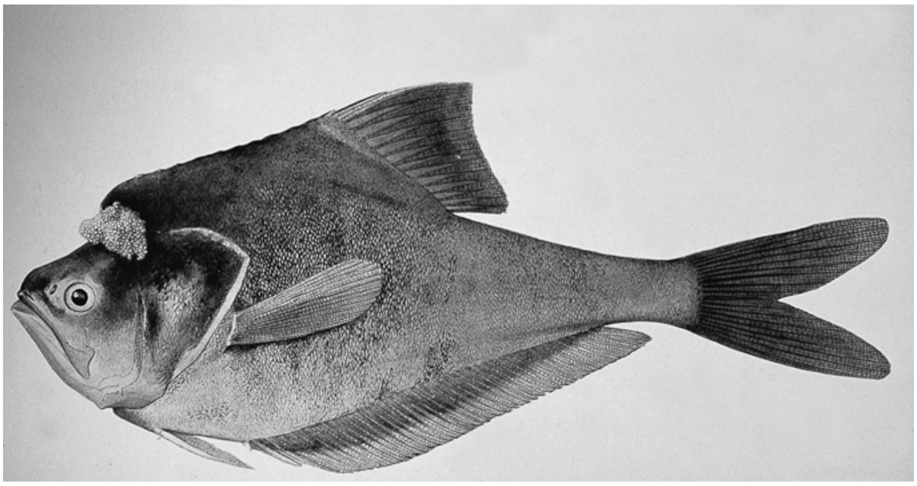


Figure 2. Male *Kurtus gulliveri* with egg mass (from Berra 2001, after Weber 1913, plate 13, figure 3).

the hook thickens distally and, in some specimens, may form nearly a complete eyelet for the attachment of the egg mass (Figure 4a). During the course of fieldwork for this study, some males with red, blood-engorged hooks were taken from the gillnets (Figure 4b,c). Although the hooks may have been inflamed from being caught in the gillnet, the edematous hooks led to the hypothesis that they provide oxygen and/or nutrition to the egg mass. This paper describes the histology of the skin and hook and investigates that possibility.

Methods and materials

The Adelaide River is a large, turbid, tropical river system strongly influenced by two high and two low tides each day. Tidal variation at the mouth can be as

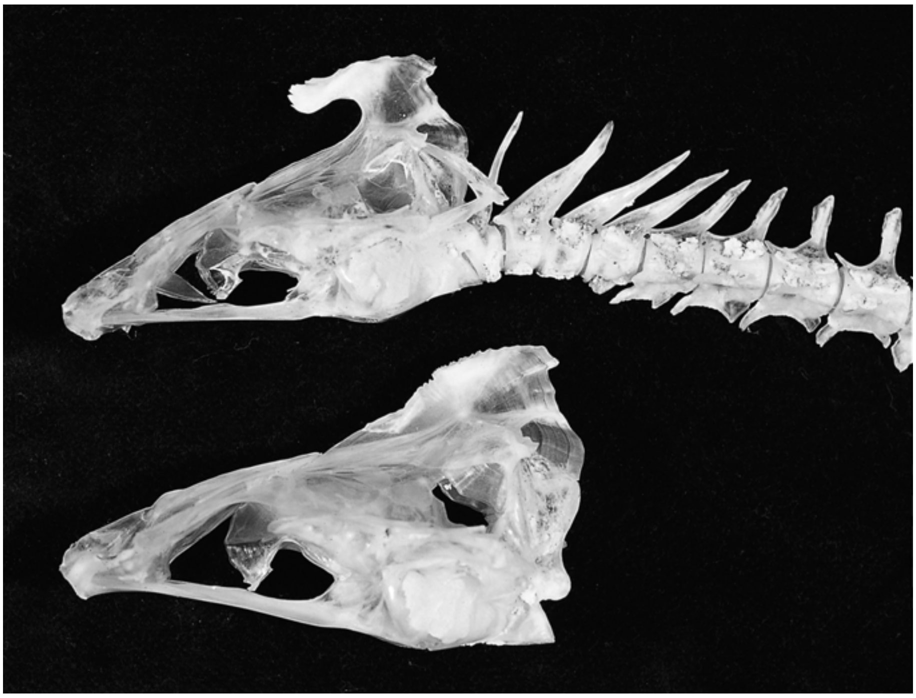


Figure 3. Skull of male *Kurtus gulliveri* (224 mm SL, top) and female (280 mm SL, bottom) showing the osteological basis of the male's hook and the well developed supraoccipital crest of both sexes.

much as 7 m. The study area was described by Berra & Wedd (2001). Specimens for this study were taken from Marrakai Creek (12°42′S 131°19.7′E), a freshwater tributary 82.1 km upstream from the mouth of the Adelaide River. The river banks are mudflats dominated by mangroves. Messel et al. (1979, figure 3.2, p. 13) described the ecological conditions and provided a map of the river. The wet season, when the river overflows its banks and spills into adjacent wetlands and flood plains, usually runs from December to April, and the dry season lasts from May to November.

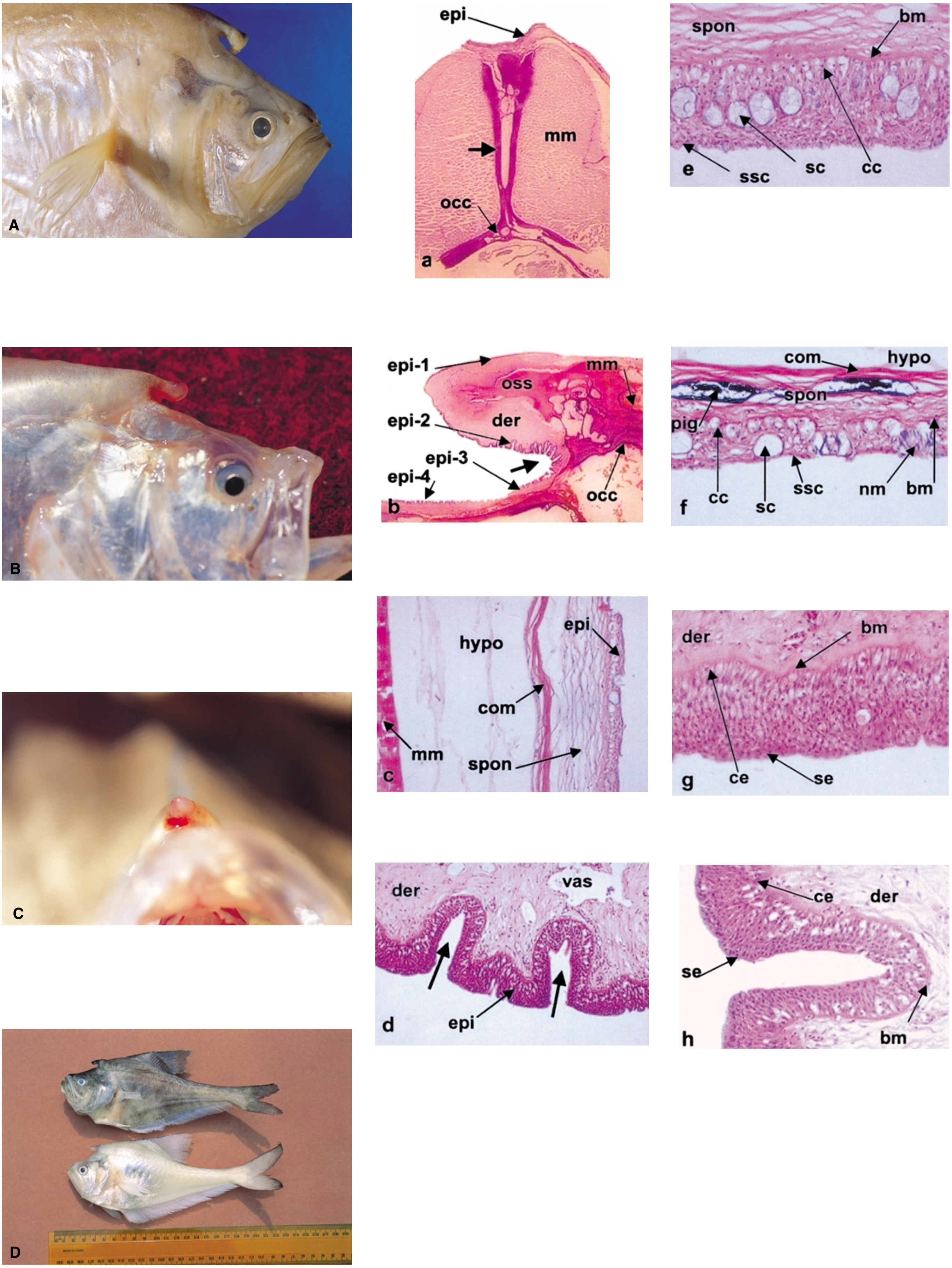
Nurseryfish were collected with 10 and 13 cm mesh gill nets that were about 2.5 m deep and up to 24 m long. Large fish were placed on ice immediately and preserved a few hours later in 10% formalin in the laboratory. Smaller specimens were placed in 10% formalin immediately. Live specimens were transported to a 5000 liter aquarium at the Territory Wildlife Park near Darwin. The only way we could hope to learn how the eggs become attached to the male's hook would be to observe captive breeding. The large population of saltwater crocodiles, *Crocodylus porosus*, made direct observation by divers too dangerous (Grzelewski 2001), and the river is too turbid for remote video observation.

Tissues of representative mature male without adherent egg mass, mature female and immature nurseryfish were fixed in 10% buffered formalin for histological examination. Bony elements were decalcified in formic acid/sodium citrate. Tissues were embedded in paraffin and sectioned at 5 microns. Tissue sections were stained routinely with haematoxylin and eosin and the periodic acid Schiff reaction was used to demonstrate mucopolysaccharides (Culling 1976).

Results

Captive specimens and skin pigment

Nurseryfish are a delicate species and proved difficult to keep in captivity. They have a heavy coating of mucus and capture by gill net resulted in the large-scale shedding of strings of mucus even in lightly netted specimens. They did not feed readily, and most died in less than one month. Knowledge of courtship and spawning behavior will have to await further developments in husbandry. Some specimens became heavily pigmented within 1–2 weeks of captivity and developed a charcoal color instead of the usual silvery color of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freshly caught specimens (Figure 4d). This may have been a protective response to the relatively dim illumination on the clear tank water as opposed to the very turbid waters in which nurseryfish normally live. Pigment-stained liquid was visible in white enamel dissecting pans when dark specimens were removed from the pans. Although the pigment-stained liquid was not examined, it was noted that the integument appeared fragile and readily desquamated on capture or handling.

Morphology of the hook of the nurseryfish

Mature male nurseryfish possess a unique morphological feature, a characteristic anterior bulbous protrusion in the dorsal midline of the head that forms a well-defined 'hook' (Figure 1). This hook appears to play a critical and unique role in the capture, brooding, and possible nurturing of the egg mass of the female. No other teleost fishes display such a structure. Males of the Asian species, *K. indicus*, have a tiny hook but are not known to carry eggs (Hardenberg 1936). This brooding behavior suggests the hook of the male nurseryfish may possess specific histological features associated with adhesion of the egg mass and/or may play a specific, undefined physiological role in addition to physical entrapment of the egg mass.

Gross morphology

Grossly, the hook is formed by an anterior extension of the supraoccipital bone (Figure 3). The bone arises as a flattened vertical protrusion in the midline of the dorsal skull (Figure 5a), extending anteriorly as an expanding shaft of bone before terminating in an irregular, somewhat bulbous projection (Figure 5b). The bony structure of the hook is invested posteriorly with subcutaneous muscle and anteriorly by collagenous dermal tissue. The epidermis, continuous with the epidermis of the head, overlies the muscle and dermal tissues (Figures 5a,b). The hook, in common with the integumentary tissues of the head, is devoid of scales, unlike the preopercle, opercle, and the body which are covered with fine cycloid scales. In longitudinal section, the anterior protrusion of the hook and the underlying dorsal surface of the head form a distinct cleft.

Sub-gross morphology

Posteriorly, the dorsal bony extension of the hook arises from the supraoccipital bone as a flattened plate of bone (Figure 3), the matrix of which contains one or more elongate cavities effectively dividing the dorsal plate. The bone is closely invested by subcutaneous skeletal

Figure 4. A – A 202 mm SL male *K. gulliveri* from the West Alligator River, Northern Territory, Australia showing the hook forming a nearly complete eyelet. Specimen S.14634-003 Northern Territory Museum. B – Lateral view of a 205 mm SL male *K. gulliveri* showing the red, edematous hook. This photo was taken immediately after capture while the fish was alive. C – Medial view of the same male from 4b. Note the highly vascularized hook and the pool of blood in the cleft of the hook. D – Two male *K. gulliveri*, both 179 mm SL. The dark specimen died in the Territory Wildlife Park aquarium after about 30 days in captivity. It was originally the same color as the lighter specimen, but darkened in captivity. Both were caught at the same time, but the lighter specimen was preserved immediately.

Figure 5 a – Low magnification view of transverse section of proximal hook, showing the vertical bony plate (arrow) arising from the supraoccipital bone (mm = skeletal muscle, occ = supraoccipital bone of skull, epi = epidermis). b – Low magnification, longitudinal view of mid-line of hook showing bony and integumentary tissues extending anteriorly from the supraoccipital bone (occ), forming a distinct cleft with the underlying head (arrow). Note the central bony matrix (oss) inter-digitating anteriorly with the dermal tissues (der) (mm = skeletal muscle, epi-1 = flattened dorsal epidermis of hook, epi-2 = rugose epidermis of dorsal aspect of cleft of hook, epi-3 = rugose epidermis of ventral aspect of cleft of hook, epi-4 = flattened dorsal epidermis of head). c – Sub-gross morphology of the integument and underlying musculature of the dorsum of the hook (epi = flattened epidermis, mm = skeletal muscle, hypo = hypodermis, com = stratum compactum of dermis, spon = stratum spongiosum of dermis). d – Sub-gross morphology of the cryptoform integument in the cleft of the hook. Arrows denote distinct pits or crypts (epi = epidermis, vas = vascular or lymphatic space, der = collagenous dermal tissues). e – Structure of the epidermis of the integument overlying the dorsal surface of the hook (spon = stratum spongiosum of the dermis, bm = basement membrane, cc = clear cells, sc = secretory cells, ssc = stratified cells of epidermis). f – Structure of the non-keratinised, stratified squamous epidermis of the integument overlying the head of the nurseryfish (spon = stratum spongiosum of the dermis, com = stratum compactum of the dermis, bm = basement membrane, cc = clear cells, sc = secretory cells, ssc = stratified cells of epidermis, pig = pigment-containing chromatophore, hypo = hypodermis, nm = neuromast). g – Flattened integument approaching confines of the cleft of the hook. Note the non-keratinising, transitional epithelium with columnar cells lining basement membrane and superficial squamous cells. Note absence of secretory or other specialised cells (ce = columnar epithelial cells, se = squamous epithelial cells, bm = basement membrane, der = dermis). h – Integument of cleft of hook showing cryptoform indentations lined by a non-keratinising, transitional epithelium devoid of secretory and neurosensory cells (se = squamous epithelial cells, ce = columnar epithelial cells, der = dermis, bm = basement membrane).

muscle in this region (Figure 5a). The skeletal muscle is overlaid by the hypodermis, a fine network of loosely organised fibers. The muscle clearly inserts onto the lateral aspect of the bony projection arising from the occipital bone, and it appears to originate from the dorsal musculature of the anterior epaxial muscles. The collagenous dermis, continuous with the dermis of the integument of the skull, lies between the hypodermis and the epidermis. The *stratum compactum* of the dermis, a thin, dense, relatively avascular layer of collagenous fibers overlies the hypodermis, while the *stratum spongiosum*, a thicker, loosely arranged, more vascular layer of collagen fibers separates the *stratum compactum* from the overlying, flattened stratified epidermis (Figure 5c). Pigmented chromatophores are randomly distributed at the interface between the dermis and hypodermis. The structure of the integument in this area is consistent with the typical teleost integument (Roberts & Ellis 2001).

Anteriorly, associated with the bulbous termination of the hook, the bone contains ovoid to irregular cavities of varying size within the osteoid matrix (Figure 5a,b). In some cases, loose connective tissue and vasculature are present in the cavities. At its anterior periphery, the bone of the hook has a series of osseous protrusions which interdigitate with the investing dermis. The bone is progressively invested anteriorly by an expanse of collagenous dermal tissue and skeletal muscle becomes progressively absent. Considerable pigmentation may occur in the sub-epidermal tissues in this area. A stratified epidermis overlays the dermis. The epidermis and underlying dermis display considerable structural diversity over the surface of the distal termination of the hook and the cleft it forms with the underlying surface of the head whereby a series of deep crypts are formed within the general confines of the cleft (Figure 5d).

Histology

Bone

The bony substance of the hook comprises a matrix of acellular, lamelliform layers of dense osteoid arising from the ossified or partially ossified cartilage of the occipital bone of the skull and may have a distinct layer of osteoblasts at its periphery. This acellular bone is typical of teleost fishes (Roberts & Ellis 2001).

Dermis

The dermis at the distal termination of the hook is not clearly divided into *stratum compactum* and *stratum spongiosum*, but progressively arises from these layers posteriorly. The dermis comprises a matrix of collagen fibers and fibrocytes, more dense near the bone, becoming loosely organised and fibrillar towards the epidermis. Numerous capillaries and small arterioles and venules are present, especially subtending the epidermis. The dermis immediately associated with the crypts is highly vascularized. Numerous thin-walled, endothelium-lined vessels devoid of erythrocytes are present, many of which may represent lymphatics (Figure 5d). Occasional mononuclear and lymphocytic cells are distributed in the dermis.

Epidermis

The epidermis of the integument approaching and within the confines of the cleft of the hook differs substantially from the epidermis of the integument of the head and other regions of the body. The epidermis overlying the majority of the head and other body regions is of similar morphology (Figure 5c,e) and representative of typical teleost epidermis (Roberts & Ellis 2001). The principle cell is the malpighian cell which forms a stratified, non-keratinising epithelium. Within this epithelium are numerous specialised cells including large globular secretory cells and more basilar clear cells while in some areas, neurosensory cell accumulations are present (Figure 5e,f).

A relatively sharp transition occurs between the typical teleost epidermis and the epidermis approaching the confines of the cleft of the hook. Here, malpighian cells form a stratified, non-keratinising epithelium devoid of secretory cells. The basal layer appears columnar in shape, retaining cells with clear vacuoles in the basal layer as elsewhere. The cells become progressively cuboidal then squamous near the surface (Figure 5g). This epithelium continues into and lines the crypts in the integument in the depths of the cleft of the hook (Figure 5h).

Discussion

The integument investing the hook shows marked regional variations in structure. The integument on the dorsal, dorso-lateral and antero-dorsal surfaces of the hook is essentially flattened, overlying the dermis and contiguous with, and resembling the integument of the head and other body surfaces. The epithelium of this portion of the integument is typical in structure of teleost fishes, being principally stratified squamous in nature and containing numerous specialised secretory cells and neuro-sensory cells. The integument approaching the confines of the cleft of the hook, i.e., the anterior and antero-lateral surfaces of the hook becomes increasingly indented in nature while those surfaces within the confines of the cleft of the hook contain numerous, distinct, cryptoform invaginations extending into the underlying dermis. The stratified epithelium investing this area of the integument is distinctly different, characterised by a columnar basal layer differentiating as squamous cells at the surface. There is a distinct absence of mucus secretory and neuroepithelial cells. The presence of intact epithelium in the cleft of the hook suggests that gill netting did not abrade the hook area and produce the edema seen in some specimens (Figure 4b,c).

Male egg carrying and external skin brooding occur in several unrelated fish families (Clutton-Brock 1991). In the Syngnathidae, male pipefish have either uncovered or enclosed ventral brood pouches to which the eggs are glued, and male seahorses have a marsupium in which the eggs are deposited by the female (Jones & Avise 2001). A placenta-like connection allows the male to transfer nutrients to the embryos (Berglund et al. 1986, Haresign & Shumway 1981). Some female South American catfishes (Aspredinidae) develop vascularized, transient outgrowths of the integument (cotylephores) on their abdominal surface to which the eggs are attached. Female ghost pipefish, *Solenostomus* (Solenostomidae), develop cotylephores within their fused pelvic fins. These structures are thought to function in gas exchange as well as egg attachment in both families (Wetzel et al. 1997).

Speculation

The cryptoform nature of the integument within the confines of the nurseryfish's hook and the absence of secretory cells suggest a specialised adaptation facilitating adhesion of the sticky egg mass of the female. Furthermore, it is possible that the highly vascularized dermis of the hook plays a role in gaseous exchange and/or nutrition of the egg mass. Only physiological measurements on 'pregnant males' in captivity can confirm this hypothesis. Engorgement with blood in the stratum spongiosum of the hook's dermis may help clamp the egg mass in place.

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