

Preliminary contribution to the knowledge of the sensory development and apparatus of the dusky grouper *Epinephelus marginatus* (Lowe, 1834)

*Contribution préliminaire à la connaissance de l'équipement
et du développement sensoriels du mérou
Epinephelus marginatus (Lowe, 1834)*

Clara Boglione*, Claudio Selmo*, Monica Contegiacomo**, Maria Teresa Spedicato**, Stefano Cataudella*

*Lab. Exp. Ecol. & Aquac., Department of Biology, University of Tor Vergata,
Via della Ricerca Scientifica 430, 00133 Rome, Italy
e-mail: boglione@uniroma2.it

**COISPA Tecnologia & Ricerca, C.P. 62, 70042 Mola di Bari, Italy
e-mail: coispa@eostel.it

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Mots clés : mérou, développement larvaire, organe olfactif, neuromaste.

ABSTRACT

Boglione C., C. Selmo, M. Contegiacomo, M.T. Spedicato, S. Cataudella, 1999 - Preliminary contribution to the knowledge of the sensory development and apparatus of the dusky grouper *Epinephelus marginatus* (Lowe, 1834). Mar. Life, 9 (1) : 9-17.

This is a study on the sensory apparatus of the larval and juvenile dusky grouper. Preliminary information on grouper larvae feeding behaviour, an aspect previously lacking in the literature, was indirectly gathered. Scanning electron microscopy (SEM) investigations were carried out on experimental larvae obtained by hormone induced spawning and on one juvenile collected from the wild. In the experimental trials, larvae survived 42 days in 1996 and 51 in 1998. At hatching, 2 olfactory placodes and 7 neuromasts were differentiated. At days 3-4, eyes were pigmented, and 9-10 free neuromasts were differentiated on the head and 1-2 on the middle line of the trunk, on each side. The number of neuromasts slowly increased and at day 42, a maximum of 7 on the trunk, on each side, and 27 on the head were observed. Up to day 42 from hatching, the olfactory organ was still represented by two external pits. No taste buds were observed. The wild juvenile showed an olfactory rosette composed of 18 lamellae. The rostral region of the head showed a central chemoreceptive region and two lateral mechanoreceptive areas. Numerous taste buds were scattered on the rostral head, on the lips, the inner mouth and dorsal pharynx. The authors hypothesise that dusky grouper larvae identify food mainly by sight while juveniles can rely also on chemo-receptive inspection.

RÉSUMÉ

Boglione C., C. Selmo, M. Contegiacomo, M.T. Spedicato, S. Cataudella, 1999 - [Contribution préliminaire à la connaissance de l'équipement et du développement sensoriels du mérou *Epinephelus marginatus* (Lowe, 1834)]. Mar. Life, 9 (1) : 9-17.

Ce travail préliminaire a pour but d'enrichir, par rapport au manque de données de la littérature, les connaissances sur l'appareil sensoriel de la larve et du juvénile de mérou brun impliqué dans le comportement trophique. Des observations en microscopie électronique à balayage (MEB) ont été effectuées sur des larves expérimentales obtenues par induction hormonale de reproducteurs sauvages et sur un juvénile sauvage. Les élevages expérimentaux se sont achevés le 42^e jour en 1996 et le 51^e jour en 1998, par la mort des toutes les larves. A l'éclosion, la placode olfactive et sept neuromastes sont développés. A trois-quatre jours, les yeux sont pigmentés, neuf ou dix neuromastes libres sont différenciés sur la tête et un ou deux sur la ligne médiane du corps, de chaque côté. Le nombre de neuromastes augmente très peu : jusqu'au 42^e jour, on ne peut en observer que sept sur le corps, de chaque côté, et 27 sur la tête. Aucun bouton gustatif n'a été observé. Le juvénile sauvage montrait une rosette olfactive composée de 18 lamelles. La région rostrale de la tête montrait une région chémoréceptive centrale et deux mécanoréceptives latérales. De nombreux boutons gustatifs sont répandus sur la région rostrale de la tête, sur les lèvres, l'intérieur de la bouche et sur le pharynx dorsal. Les auteurs supposent que les larves de mérou peuvent localiser les proies surtout à la vue, pendant qu'elles demeurent dans la colonne d'eau, tandis que les juvéniles utilisent aussi le mécanisme de chémoréception.

INTRODUCTION

The artificial propagation of the dusky grouper (*Epinephelus marginatus*) is currently limited by a variety of constraints: the difficulty in broodstock management (proterogynous hermaphroditism with late sexual maturation); the lack of knowledge of the behaviour of larvae and juveniles; the relatively small size of grouper larvae. The first attempts at artificial reproduction allowed for a description of embryonic and early larval stages (Glamuzina *et al.*, 1998; Spedicato *et al.*, 1998, 1999a; Marino *et al.*, in press). Spartà (1935), Lo Bianco (1969), Zabala *et al.* (1997) and Dantart *et al.* (1999) provided some information on the development of wild *E. marginatus* eggs and larvae.

The scanty information on larval trophic ecology and the small size of the larval mouth (89 - 140 μm on day 8 after hatching; Spedicato *et al.*, 1998, 1999a) still represent the main limit to standardising seed production. So this study focuses on the ontogenesis of chemical and mechanical receptors to provide a preliminary contribution to the study of larval feeding ecology.

MATERIAL AND METHODS

Two different egg depositions were induced by LHRH-a stimulated females and 1 sex-reversed male in 1996 (Spedicato *et al.*, 1999b, 1999c) and 1998 (Spedicato *et al.*, 1998). The rearing protocol follows Spedicato, Boglione (2000).

Larvae (1 - 5 specimens collected daily until day 51), and one wild juvenile (weight: 155 g; total length: 21 cm) were fixed in glutaraldehyde (2.5% in 0.1M phosphate buffer, pH 7.2), postfixed with tetroxide osmium, critical point dried and gold coated with AGAR Sputter Coating. Scanning electron microscopy (ZEISS DSM 950) observations were performed on the larvae (till day 42) and the wild juvenile.

RESULTS

The average total length (TL) of the newly hatched larvae was 2.03 mm (CV = 5.9%), The hatching rate in both trials was about 65% and severe mortality was recorded on days 6, 8 and 12 after hatching. In the 1996 experiment, only 1% of larvae survived after day 12, and none after day 42. In the 1998 production, only few larvae survived after day 30 and the last one died on day 51.

The main developmental stages until day 42 from hatching (1996 experimental trial) are summarised in figure 1, whilst the increment of free neuromasts is reported in table I.

SEM observations revealed the presence of free neuromasts (figure 2) in just-hatched larvae on the rostro-dorsal region (1 couple), and ventrally (1) and caudally (1) to the optic vesicle, on the trunk (1). Olfactory buds were differentiated at hatching (diameter: 8 μm) and ciliated sensory cells, microvillate supporting cells and ciliated non-sensory cells were observed.

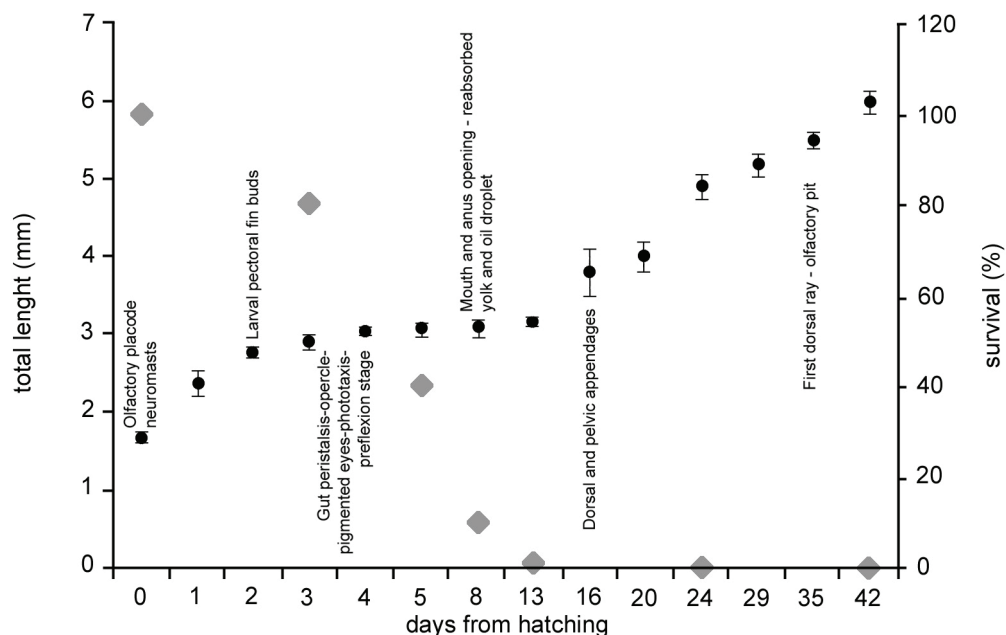


Figure 1 - Survival rate (%): ◆, total length (mm): ● and main developmental stages of dusky grouper larvae. Data referred to 1996 experimental reproduction.

Taux de survie (%) : ◆, longueur totale (mm) : ● et principaux stades de développement larvaire du mérou. Données issues de la reproduction expérimentale de 1996.

Table I - Number of free neuromasts observed in experimental dusky grouper larvae. Age: days from hatching; TL: total length (mm); rostro-dors.: rostro-dorsal region of the head; rostro-ventr.: rostro-ventral region of the head; orb.: orbital; trunk: trunk lateral line; (*): notochordal length (mm); (¹): numbers referred to left side of the body. / Nombre de neuromastes observés chez la larve de mérou brun. Age : en jours ; TL : longueur totale (mm) ; rostro-dors. : région rostro-dorsale de la tête ; rostro-ventr. : région rostro-ventrale de la tête ; orb. : orbital ; trunk : ligne latérale ; (*) : longueur notochordale (mm) ; (¹) : données correspondant au côté gauche du corps.

Age	n°obs.	TL	rostro-dors.	rostro-ventr.	antero-orb. ¹	retro-orb. ¹	supra-orb. ¹	sub-orb. ¹	trunk ¹
1	2	1.7-2.0	1x2	0	0-1	1	0	1	1
2	4	1.8-1.9	1x0/2	0	0	0-1	0-2	0-2	0-2
3	4	2.0	1x2	0	0-1	0	2	2	0-3
4	4	1.6-2.1	1x2	0	0-1	0	2	0-1	1-2
5	2	2.0	2x2	0	0	1	0	1	2
6	2	?	0	0	2	0	2	1	2
7	2	1.6	2x2	1	0-2	0	2-3	2	1
8	2	1.8	2x2	1x2	1-3	0	1-2	2	1
16	1	2.6	2x2	0	5	0	1	2	4
20	1	2.6 (*)	2x2	1x2	5	0	1	2	4
24	1	3.3	2x2	0	5	2	2	2	4
29	1	3.2	0	0	5	2	2	3	0
35	1	2.9	2x2	0	4	0	2	3	4
42	1	3.3	2x2	2x2	5	0	3	2-3	7

By days 3 and 4, eyes were pigmented and larvae preferred the shaded areas of the tank. The mouth was open in all the larvae by day 3.

Free lateral line neuromasts were mainly present on the lateral and dorsal sides of the head. During the larval development, their number slowly increased (table I), forming central clusters (rostro-dorsal and rostro-ventral ones) or bilateral lines (antero-orbital, retro-orbital, supra-orbital, sub-orbital).

Larvae which were more than 16 days old showed one elongated dorsal and two pelvic rays, with a melanophore patch on the upper part. At this stage surviving larvae showed very active predatory behaviour. Larvae utilise the elongated fin rays to perform vertical movements (by cephalad or caudad movements of the dorsal spine), or to change (left/right) swimming direction (ventral spines).

The olfactory organ elongated in a rostro-cau-

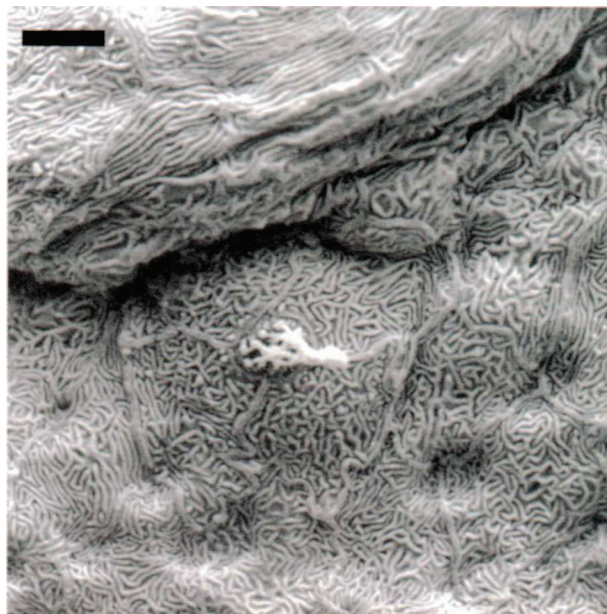


Figure 2 - Four day-old larva: sub-orbital free neuromast of the cephalic lateral line system. Bar = 10 µm. / Larve âgée de 4 jours : neuromaste libre de la ligne latérale céphalique situé dans la région sub-orbitaire. Barre = 10 µm.

dal direction (figure 3) assuming an oval shape (major diameter = 70 μm). The beginning of the deepening process of the olfactory placode (olfactory pit) was observed starting from day 35, but none of the experimental larvae showed the completion of the process. At day 42, the largest diameter of the olfactory organ was 83 μm .

At day 42, a maximum of 27 free neuromasts was found on the head and 7 on the middle line of each side of the trunk.

Neither outer nor inner taste buds were observed until day 42.

On day 51 the last larva (TL=14.7 mm) died. It still displayed elongated fin rays, a transparent body with a small melanophore placed in the middle part of the caudal peduncle and no scales. The flexion of the notochord had been attained, as well as dorsal, anal and pelvic fin formation. At this stage, the dorsal fin elongated ray constituted the second element of the dorsal fin and the pelvic ones the first rays of the pelvic fin. The anal fin had three thin spines, the second one being longer than the other ones. On the second, third and fourth dorsal, the first pelvic and the second anal elongated rays, many thin and sharp protuberances were lined up in 2-4 longitudinal rows. No sensorial cells were observed on elongated rays.

The wild juvenile showed two nares, the anterior one was tubular, with an epidermal flap (valve) (figure 4a). The olfactory organ was composed of 18 primary lamellae radiating from a central ridge (raphe, figure 4b), which arises rostro-caudally from the floor of the nasal cavity, to form a rosette-like pattern (type F, according to Yamamoto, 1982). With the exception of margins proximal to the raphe, each lamella was covered by the olfactory epithelium

(figure 4c), containing ciliated sensory cells, microvillate supporting cells, ciliated non-sensory cells and mucous cells (but no microvillate sensory cells; figure 4d).

On the head, on the rostral dorsal surface, a double row of lateral line pore canals delimited a central chemoreceptive region (with elevated taste buds) from bilateral mechano-receptive areas (figure 5). On the rostral ventral part, one centrally coupled row of lateral line pore canals extended laterally and caudad, along with the dentale profile. The area delimited by the frontal rows contained many scattered taste buds (figure 6). Free neuromasts without evident muco-polysaccharidic cupulae still persisted all around the nares (figure 7). In the mouth, many thin teeth were dispersed on pre-maxillar (figure 8a), dental (figure 8b), palatin and pharyngo-branchial organs, most of which were still covered by an epithelium (figure 8b). Taste buds were numerous, elevated and scattered among the teeth, tongue (figure 9, a and c), on the lips (figure 9a), the buccal floor and the pharyngo-branchial organ. They seemed to be at different differentiation stages (figure 9, a and b).

DISCUSSION

The two tentative artificial reproductions carried out in 1996 and 1998 did not result in the production of grouper juveniles and the precocious death of larvae at maximum at day 51 post hatching revealed inappropriate rearing conditions. Consequently, the possibility that the observations we carried out could be inherent to larvae in sub-optimal conditions should be considered. On the other hand, there is a total absence of information on the senso-

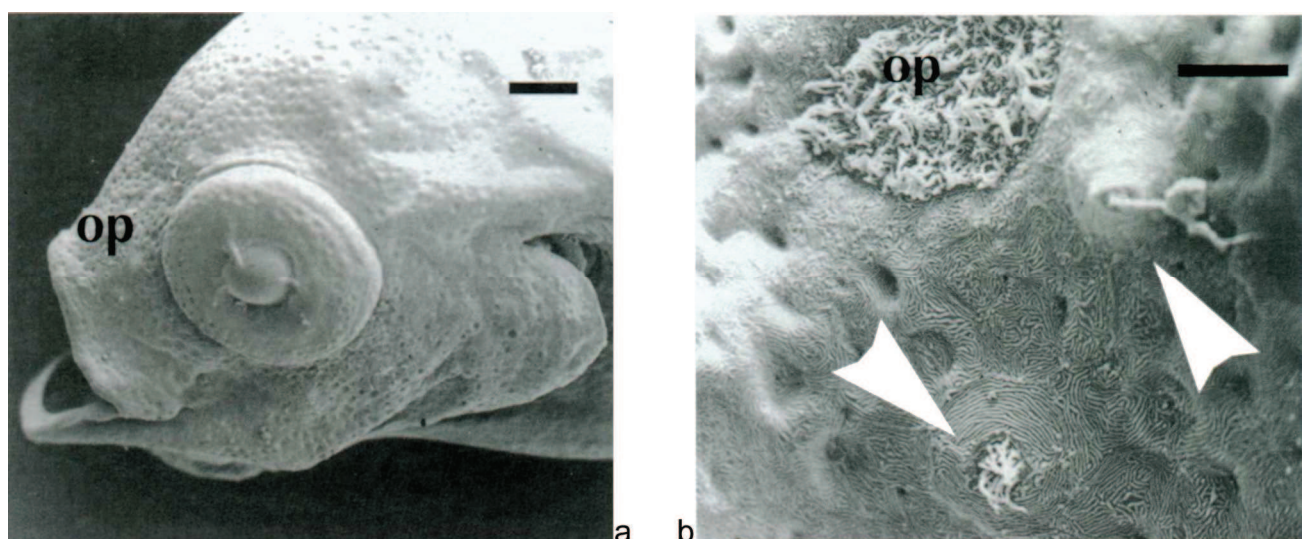


Figure 3 - Twenty four day-old larva. (a): head profile (op: olfactory placode). Bar = 100 μm . (b): particular of photo (a) magnifying the external olfactory organ (op). Arrows indicate two free neuromasts. Bar = 20 μm . / Larve âgée de 24 jours. (a) : silhouette céphalique (op = placode olfactive). Barre = 100 μm . (b) : détail de la photographie (a) montrant l'organe olfactif externe (op). Les flèches indiquent deux neuromastes libres. Barre = 20 μm .

ry equipment of this species. Further studies are therefore necessary to validate our observations.

Both experimental reproductions indicate a slow achievement of adult characteristics in dusky grouper larvae. On day 51 after hatching, larval dorsal and pelvic appendages still persisted, as well as the transparency of the body and the absence of scales. Also the wild individual showed an uncompleted arrangement of emerged teeth (if compared with the number in adult) and taste buds at different differentiation stages.

As regards the larval sensorial apparatus, it is

well known that lateral line organs (neuromasts) are involved in mechanoreception: in particular, canalised neuromasts perceive water accelerations while free neuromasts detect rheotaxis. As neuromasts did not canalise in the observed experimental larvae, these should be considered as being capable of detecting rheotaxis, but not water accelerations. Furthermore, larvae showed a quite high density of free neuromasts on the head, particularly in the rostro-dorsal area. According to Webb (1989), this may indicate an activity in the water column and not on the bottom.

Despite the absence of taste buds until day

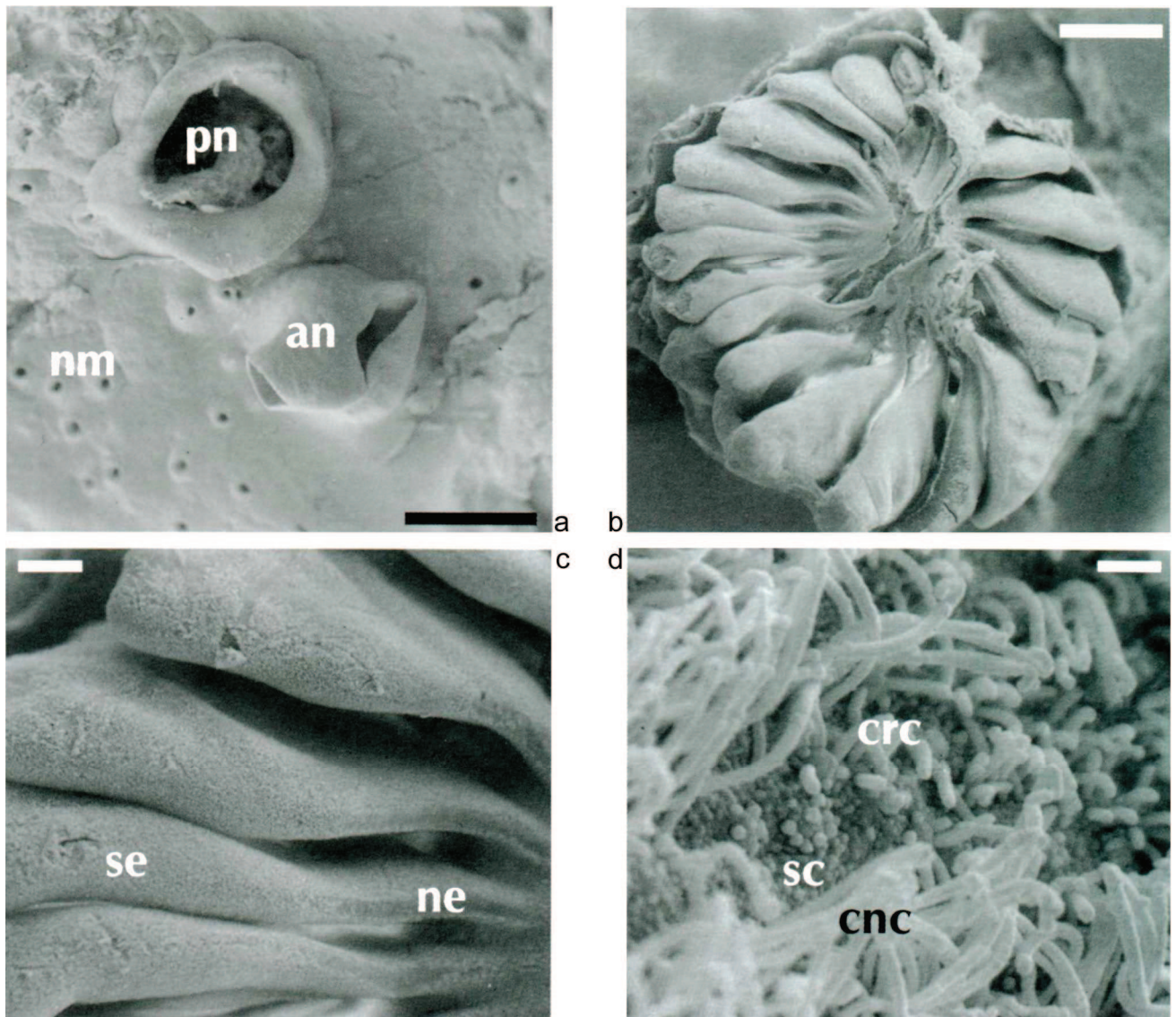


Figure 4 - Wild juvenile. (a): anterior (an) and posterior (pn) nares (nm: neuromast). Bar = 1 mm. (b): olfactory rosette. Bar = 500 μ m. (c): olfactory lamellae. The sensitive epithelium (se) covers the entire lamella with the exception of the dorsal margin, proximal to the raphe (ne). Bar = 100 μ m. (d): olfactory epithelium (crc: ciliated receptor cells; sc: supporting cell; cnc: ciliated non-sensory cell). Bar = 2 μ m. / *Juvenile sauvage.* (a) : narines antérieure (an) et postérieure (pn) (nm : neuromaste). Barre = 1 mm. (b) : rosette olfactive. Barre = 500 μ m. (c) : lamelles olfactives. L'épithélium sensoriel (se) recouvre toute la lamelle, à l'exception du bord dorsal qui est proche du raphé (ne). Barre = 100 μ m. (d) : épithélium olfactif (crc: cellule ciliée réceptrice ; sc : cellule de soutien ; cnc : cellule ciliée non sensorielle). Barre = 2 μ m.

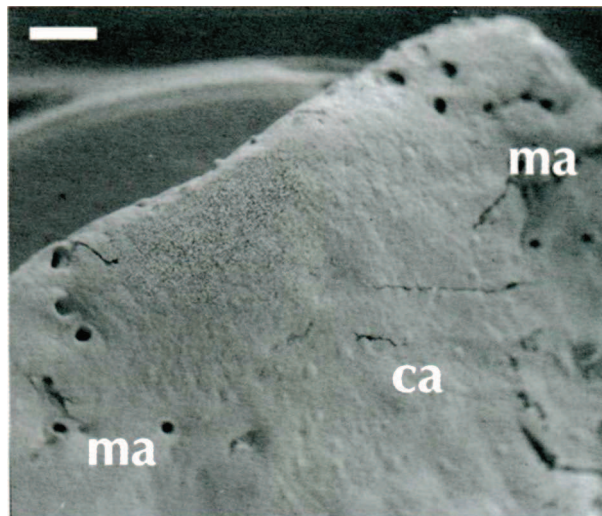


Figure 5 - Cephalic tegument of a wild juvenile: the rostro-dorsal region is divided by a coupled row of pore canals of the cephalic lateral line system in a central chemoreceptive area (ca), containing many elevated taste buds, and two lateral mechano-receptive areas (ma) with both free and pore-engaged neuromasts. Bar = 500 μ m.

Surface céphalique d'un juvénile sauvage : la région rostro-dorsale est partagée par une double ligne de canaux à pores de la ligne latérale céphalique, en une région centrale chémo-réceptrice (ca comprenant de nombreux boutons gustatifs élevés et deux régions latérales mécanoréceptrices (ma) avec neuromastes libres ou engagés dans les pores. Barre = 500 μ m.

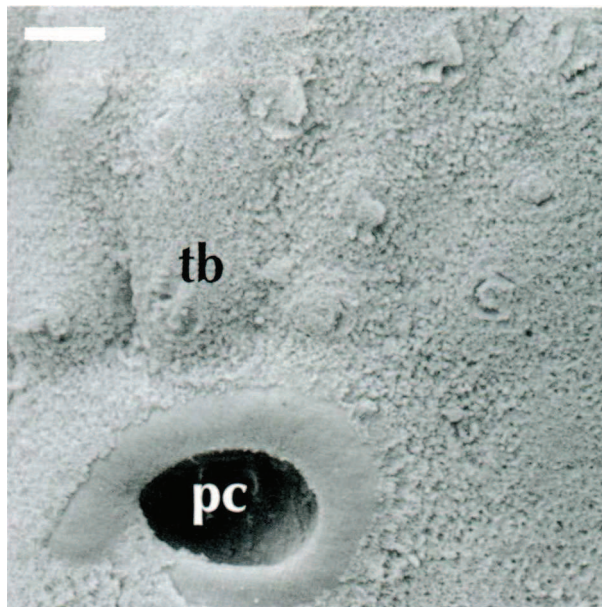


Figure 6 - Wild juvenile rostro-ventral region characterised by a central chemoreceptive area (tb: taste buds) and by lateral mechanoreceptive areas (pc: pore canal). Bar = 100 μ m. / Région rostro-ventrale d'un juvénile sauvage caractérisée par une zone centrale chémo-réceptrice (tb : bouton gustatif) et des zones latérales mécanoréceptrices (pc : canaux à pores). Barre = 100 μ m.

42, chemoreception could be effected in dusky grouper larvae by the olfactory organ and by free neuromasts. The latter are in fact capable of chemoreception, as some authors have indicated. According to Katsuki and Yanagisawa (1982), free neuromasts respond to various metallic ions in fish and tadpoles. Yamashita (1982) reported that whilst taste buds responded in *Pseudorasbora parva* to all four basic tastes, free neuromasts responded to acids and

salt solutions. In the wild dusky grouper juvenile, the olfactory organ is constituted by 18 primary lamellae, radiating in all directions from a raphe (F arrangement), with a continuous sensory epithelium except for the proximal dorsal margin of the lamella and with an extremely dense distribution of non-sensory cilia. In *E. septemfasciatus*, Yamamoto (1982) found 23 lamellae with the same F arrangement and density of non-sensory cilia but with a

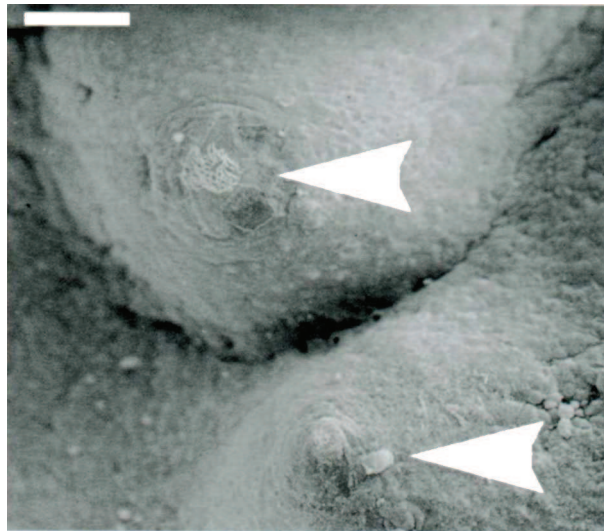


Figure 7 - Wild juvenile: neuromasts around (arrows) the nares are free (superficial), and placed on the apex of epidermal papillae. Bar = 50 μ m. / Juvénile sauvage : neuromastes (flèches) libres (superficiels) autour des narines, placés au sommet de papilles épidermiques. Barre = 50 μ m.

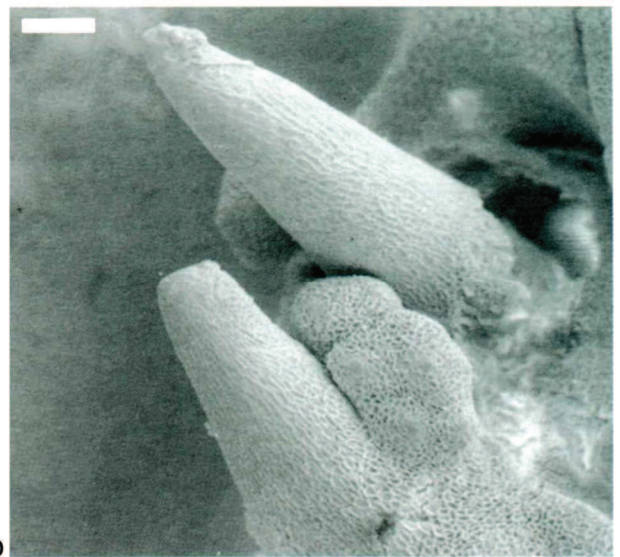


Figure 8 - Buccal cavity of the wild juvenile: teeth of different sizes are present on the pre-maxillar, dentale, palatin and pharyngo-branchial organ. (a): teeth on pre-maxillar. Bar = 200 μ m. (b): teeth on dentale still covered by epithelium. Bar = 100 μ m. / Cavité bucale du juvénile sauvage : des dents de différente taille sont situées sur le prémaxillaire, le dentaire, le palatin et sur l'organe pharyngo-branchial. (a) : dents du prémaxillaire. Barre = 200 μ m. (b) : dents du dentaire souvent couvertes par l'épithélium. Barre = 100 μ m.

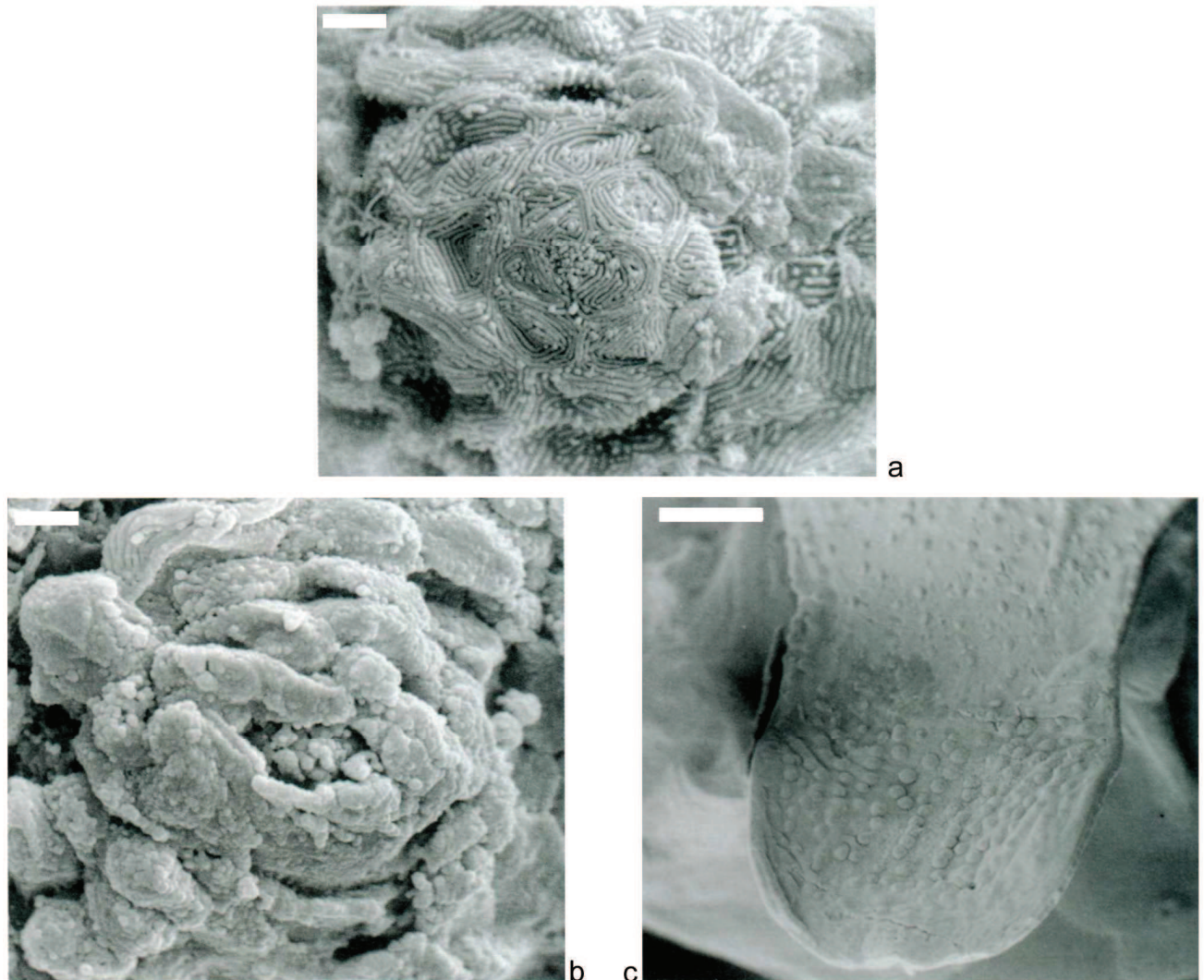


Figure 9 - Taste buds in wild juvenile. (a): taste bud of II Reutter' type (Reutter, 1973) observed on the tongue. Bar = 5 μ m. (b): I type taste bud photographed on lower rim. Bar = 5 μ m. (c): distribution of elevated taste buds on tongue (*copula ioidea*). Bar = 1 mm. / Bourgeons gustatifs chez le juvénile sauvage. (a) : papille gustative du deuxième type selon Reutter (1973) observée sur la langue. Barre = 5 μ m. (b) : bourgeon gustatif du premier type selon Reutter photographié sur la lèvre inférieure. Barre 5 μ m. (c) : distribution des papilles gustatives sur la langue (*copula ioidea*). Barre = 1 mm.

continuous distribution of sensory epithelium except for the entire distal margin of the lamella. The difference in lamellae number was to be expected since their number and arrangement have been used as diagnostic features in the taxonomy of some teleostean groups (see Yamamoto, 1982, for a review). Some authors suggest there is a correlation between olfactory organ size or number of lamellae and the acuity of the olfactory sense (Pipping, 1927; Yamamoto, 1982). Considering this, the dusky grouper wild juvenile we observed showed quite a low number of lamellae compared to other species: in some Anguilliformes there are as many as 120 olfactory lamellae, for instance, and Salmonids present 5-10 secondary lamellae on each primary lamella. Adult sea bass (*Dicentrarchus labrax*) show 36 primary lamellae, each carrying secondary folds (Boglione *et al.*, 1988). As the number of lamellae in the adult

dusky grouper is unknown, other studies must be attempted to clarify if the low number we observed is the definitive one. According to Yamamoto (1982) and Yamamoto, Ueda (1978a-d), the olfactory ability is also related to the cellular composition of the olfactory epithelium. After analysing 80 different Teleosts, the authors suggested that fish with strong olfactory ability have continuous sensory areas with dense non-sensory cilia (as in the dusky grouper juvenile we observed), whilst fish believed to rely mainly on visual sense have dispersed sensory areas with no or sparse non-sensory cilia. So, on the basis of this indication, *E. marginatus* could rely mainly on its chemoreceptive ability for food detection during the juvenile period, owing to the continuous sensory epithelium in the olfactory organ, and the numerous taste buds and free neuromasts.

The larvae already showed light sensitivity from the 3rd - 4th day from hatching, therefore it would seem that there is a greater visual than olfactory capability in dusky grouper larvae. They use the dorsal and pelvic spines to change direction, and identify food items mainly by sight, selecting them principally by size (mechanoreceptors) and secondarily by chemoreceptors.

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