Photoperiod and feeding behavior of the Antarctic fish *Notothenia rossii* (Perciformes: Nototheniidae) and functional morphology of chemical and visual sensory structures used in foraging

Lucélia Donatti 1; Tânia Zaleski 2; Patrícia Calil 3 & Edith Fanta 1, 4

1 Departamento de Biologia Celular, Universidade Federal do Paraná, Caixa Postal 19031, 81531-970 Curitiba, Paraná, Brasil. E-mail: donatti@ufpr.br
2 Programa de Pós-graduação em Zoologia, Departamento de Zoologia, Universidade Federal do Paraná. Caixa Postal 10920, 81531-980 Curitiba, Brasil.
3 Centro Universitário Campos de Andrade. Rua Marumby 283, 81220-090 Curitiba, Paraná, Brasil.
4 In memoriam.

ABSTRACT. The influence of photoperiod on the ability of *Notothenia rossii* Richardson, 1844 to detect prey was studied experimentally. In 12L/12D there was the highest number of individuals stimulated visually and chemically while in 24D chemical perception was the same as that observed in 12L/12D, the latter being lower than in 24L. The ultrastructure of chemo- and photo-sensory structures, involved in the detection of food, was studied. The photoreceptor cells of the retina are single and double cones, and rods. Taste buds were concentrated in the central part of upper and lower lips. Their shape is even, but the size is variable. The olfactory rosette has a sensory and a non-sensory epithelium, being rich in different mucosubstances. The conclusion was that in different photoperiods there is a difference in the response to visual and chemical stimulation for prey detection and that all sensory systems are more stimulated in periods of more luminosity, mainly when there is an alternation between light and dark periods. Sensory organs are complex when compared to some other Antarctic fish such as *Gobionotothen gibberifrons* Lönnberg, 1905 and *Ophthalmolycus amberensis* Tomo, Marshoff & Torno, 1977 but similar to those of *N. coriiceps* Richardson, 1844.

KEY WORDS. Chemoreception; microscopy; photoreception.

RESUMO. Comportamento alimentar do peixe antártico *Notothenia rossii* (Perciformes: Nototheniidae) em diferentes fotoperíodos e a morfologia funcional das estruturas químico-foto sensoriais usadas na detecção do alimento. A capacidade de *Notothenia rossii* Richardson, 1844 detectar presas em diferentes fotoperíodos (24L, 24D e 12L/12D) foi estudada em tanques experimentais. Em 12L/12D houve um maior número de indivíduos estimulados visual e quimicamente enquanto que em 24D a percepção química foi a mesma que a observada em 12L/12D, sendo este último inferior a 24L. A morfologia das estruturas químico-foto sensoriais, envolvidas na detecção do alimento, foi estudada. As células fotorreceptoras da retina são os cones simples e duplos e os bastonetes. Os botões concentraram-se na região central dos lábios superior e inferior. Seu formato é constante, mas o tamanho é variável. A roseta olfatória possui um epitélio sensorial e não sensorial rico mucosubstâncias de natureza diversa. Podemos concluir que em diferentes fotoperíodos existe uma diferença de resposta na estimulação visual e química para a detecção de presas e todo o sistema sensorial são mais estimulados em períodos de maior luminosidade, principalmente quando existe uma alternância entre períodos de luz e escuro. Os órgãos sensoriais são complexos quando comparados com outros peixes antárticos como *Gobionotothen gibberifrons* Lönnberg, 1905 e *Ophthalmolycus amberensis* Tomo, Marshoff & Torno, 1977) mas similar a *Notothenia coriiceps* Richardson, 1844.

PALAVRAS-CHAVE. Fotorrecepção; microscopia; quimiorrecepção.

Feeding behaviors in many species of Antarctic fish are strongly influenced by photoperiod and light conditions in the Antarctic (BARRERA ORO & CASAUX 1990, FANTA et al. 1994, DONATTI & FANTA 2002). These different feeding strategies are also related to adaptations of the visual and chemical sensory systems (MEYER & FANTA 1998, FANTA et al. 1994, 1999, 2001, DONATTI & FANTA 2001, 2002). Through chemo and photoreception, these fish may recognize habitat features and presence of prey, preda-
tors and conspecifics (Hara 1971, 1986, Macdonald & Montgomery 1991). However, due to the seasonally limited light conditions in the Antarctic, a tendency towards dominance of mechanoreceptors exists in some species, such as nototheniids (Montgomery & Macdonald 1987, Montgomery & Combrin 1992). Also, chemoreception (including smell, taste and a general chemical sense, Hara 1971) is complex and in water, all chemosensory receptors may be stimulated by all chemicals in the water (Valentinic & Caprio 1994). Structural analyses of the retina in Antarctic nototheniids show no specific retinal adaptations for low-luminosity vision for species of shallow waters (Meyer-Rochow & Klune 1982, Eastmann 1988).

Commercial fishing in the Antarctic has increased the importance of the biology of Antarctic fish, such as N. rossii Richardson, 1844 (Tarverdyieva & Pinskaya 1972). The most economically important species are in Nototeniidae and Chaeoichthyidae (Targett 1981), which have long lives, low fecundity and slow growth compared to other cold water species (Mckenna & SAILA 1991).

Despite the Convention for the Conservation of Antarctic Marine Living Resources, and of the Protocol to the Antarctic Treaty, and their conservation measures, fishing levels have increased and are now intense for some Antarctic species (Skora & Soininski 1983, Policansky 1994). Notothenia rossii is an important species for its flesh and eggs. In the early 1970s stocks were depleted, conservation measures were adopted (Fisher & Hureau 1985) and today commercial fishing is prohibited until the stocks recover (Camer 2007). Little is known of the feeding ecology of N. rossii. Feeding habits and preferred habitats change ontogenetically (Hureau 1970, Tarverdyieva & Pinskaya 1972). It is a voracious predator (Gon & Heemstra 1990). In Admiralty Bay, while amphipods are dominant in the diet year-round, N. rossii favored salps during summer and polychaetes during the winter (Linkowski et al. 1983). In Potter Bay, gammarid amphipods were its main prey, yet other species were common in its diet (Calaux et al. 1990). In addition to amphipods, this fish also favors krill (Fanta et al. 2001).

In this context, we compared the ability of Notothenia rossii to detect prey in different photoperiods by both visual and chemical means. Also, we examined the structure of the eyes (retina) and those of chemoreception (olfactory rosette and labial taste buds). We interpret the structure of the sensory organs within the context of this species' foraging ecology to better understand feeding behavior.

**MATERIAL AND METHODS**

Notothenia rossii (n = 24) (standard length 36.3 ± 1.0 cm, mean ± S.D.) were collected in Admiralty Bay (62°09'5S, 58°26'W'), King George Island, South Shetland Islands by trammel net, line and hook. At the Comandante Ferraz Brazilian Antarctic Station, fish were acclimated in tanks under controlled environmental conditions: temperature 0.5°C ± 0.5 (mean ± S.D.), pH 7.0 ± 0.5 and salinity 36.0‰ ± 0.5. Acclimation and tests were conducted under two photoperiod treatments: 12 h light, 12 h dark (12L/12D), and 24 h light (24L) and 24 h dark (24D). Light was provided by a daylight lamp with a gray translucent filter that reduced light intensity at the bottom of the tanks to 10-15 lux. A soft red lamp and an intensity of one lux at the bottom of the tanks simulated darkness following Donatti & Fanta (1999, 2002).

Two fish in each 500 L aquarium were used to test visual or chemical stimulation. To test visual perception to detect prey, a transparent vessel filled with water was placed in a corner of the tank, with no communication of water between the two (to eliminate the possibility of chemical perception). An ~6.0 cm piece of fish (1/3 the length of the predator, Fanta & Meyer 1998) was shaken within the vessel to visually stimulate the predator. The time interval from the immersion of the fish into the vessel until the moment that the predator clearly responded was measured. To test chemical stimulation, fish extract was delivered to the aquarium through a hose. The time interval from delivery to the response by the predator was measured. Following Fanta et al. (1999, 2001) and Donatti & Fanta (2002) a 10 min maximum interval was used. Four treatments were used: 1) during light period of 12 h light, 12 h dark (12L/12D), 2) during dark period of 12L/12D, 3) 24L, and 4) 24D. Each treatment had 15 replicates for visual and chemical stimulation. Fish were not fed for 24 hours prior to each test.

Four variables were used in analyses. 1) The number of individuals that responded in any recognizable way to the stimulus, 2) the time interval until response, 3) the number of times each fish physically attacked the vessel (in the visual test) and 4) the number times each individual attacked the hose (in the chemical test). Analysis of variance was used to compare the photoperiod treatments for each of these four variables. Significance was considered when p < 0.05. (Zar 1984).

Retinas, olfactory rosettes, upper and lower lips of 20 fish were removed after decerebration. For light microscopy (LM), samples were fixed in Alfac (16 hours), dehydrated in a graded alcohol series, cleared in xylene and embedded in ParaPlast Plus®. Slides were stained with Haematoxylin and Eosin (H.E.) (Clark 1981) for general morphology and Alcian Blue pH 2.5 and pH 1.0, Periodic Acid Schiff (PAS) and Alcian Blue pH 2.5/PAS to identify mucous secreting cells (Celung et al. 1985).

For transmission electron microscopy (TEM), samples were fixed in Karnowsky (4 hours), washed in cacodylate buffer 0.2 M (pH 7.2) and post-fixed in 2% osmium tetroxide in cacodylate buffer 0.2 M (pH 7.2) for 1 hour. Material was contrasted in blocks with 2% aqueous uranyl acetate for 2 hours and embedded in Epon 812 (Lust 1961). Ultra-fine sections were contrasted in 2% aqueous uranyl acetate solution (Watson 1958) and plumbous nitrate (Reynolds 1963). Analysis was under electron microscope (JEO/LJEM 1200 EX II – Electron Microscopy Centre – Federal University of Paraná (CME-UFPFR)).

For scanning electron microscopy (SEM), samples were fixed in Karnowsky (4 hours) and washed in cacodylate buffer...
Number of fish stimulated

Fish responded to both, visual and to chemical stimuli, at all photoperiod treatments. However, treatments were similar in the number of fish that responded to the stimulus in the time interval for both visual and chemical tests (all \( p > 0.05 \)). In 24L, somewhat fewer fish responded visually \((1.33 \pm 0.51, \text{mean} \pm \text{S.D.})\) or chemically \((1.44 \pm 0.52)\). In 12L/12D, the number of individuals stimulated was the same in both the chemical and visual tests \((1.00 \pm 0.00)\). In 24D, fewer fish responded to visual stimulation \((0.66 \pm 0.57)\), but chemical perception was the same as that observed in 12L/12D \((1.00 \pm 0.00)\).

Time to response

The time to response was shorter in the photoperiod 12L/12D treatment \((51.33s \pm 44.97)\) than in photoperiod 24L \((216.16s \pm 224.50)\) and 24D \((319.33s \pm 69.06)\). Response time was also shorter for chemical stimulation in the photoperiod 12L/12D \((240.00s \pm 214.41)\), 24L \((273.66s \pm 112.48)\) and 24D \((273.83s \pm 86.94, \text{mean} \pm \text{S.D.})\). Clearly the fish responded more rapidly to chemical rather than visual stimuli, yet photoperiod had no influence on response time in either visual \((F_{2.9} = 1.87, p = 0.20)\) or chemical \((F_{2.9} = 0.9, p = 0.91)\) tests.

Number of attacks on the prey

In the visual test, the number of attacks was greater in the 12L/12D treatment \((8.66 \pm 6.02)\) than either 24L \((2.50 \pm 2.73, F_{2.9} = 5.01, p = 0.03)\) or 24D, when no attacks occurred. In the chemical test, all treatments were similar \((F_{2.9} = 1.98, p = 0.17)\), ranging from the 24L treatment \((1.11 \pm 1.42)\), 24 D \((4.50 \pm 5.68)\) and 12L/12D \((4.33 \pm 2.30)\). The prey was accepted similarly by the experimental animals using both visual and chemical cues (Figs 3 and 4). Rods have calical processes similar to those found in cones. The accessory outer segment of the rods and cones have the same origins, yet in the rod it extends directly inside the accessory outer segment and takes an eccentric route inside the limiting membrane. Ultrastructurally, the outer segment of the rods is similar to that of the cones; however, the double membranes are not continuous with the extracellular space (Fig. 4).

The cones in \(N. \ rossii\) are variable. They may be single \((1.00 \pm 0.00)\), or double \((1.44 \pm 0.52)\) or with both, single and double segments \((1.00 \pm 0.00)\). An accessory outer segment of the cone emerges from the region of the connecting cilium as a lateral process that runs along the outer segment (Fig. 6). An accessory outer segment contains an amorphous substance with a slightly granular matrix, but without biological membranes. Calical processes are not present. Muller cells have apical microvilli in the myoid region of the cones (Fig. 8). These regions of interaction among photoreceptors form the outer limiting membrane.

Ultrastructure of the olfactory rosette

A pair of olfactory organs have a variable shape, from oval to semi-spherical, in the olfactory chamber below the skin of the nostrils. The olfactory epithelium is arranged in two rows of parallel lamellae \((26 \pm 7.56)\), perpendicular to a central raphe formed by loose conjunctive tissue comprising elastic reticular fibres, collagen and blood and lymphatic capillaries. Epithelium comprises the sides of each lamella with regions having sensory and non-sensory characteristics. Epithelium with non-sensory characteristics is in the peripheral parts of the lamella and has many mucous cells. The epithelium with sensory characteristics forms a continuous layer resting on the basal mem-
brane and covers the central part of the olfactory lamellae (Figs 9-12). The olfactory epithelium comprises small spherical cells with a spherical nucleus that occupies most of the cell. They are located mainly in the intermediary and superficial region of the epithelium.

Sensory cells are bipolar neurons with long processes towards the surface that become cilia on the surface (Figs 11 and 12). They have granular endoplasmic reticulum and parallel well-developed Golgi complexes, neurotubules and mitochondria. Long axons originated from the basal regions of these neurons form the olfactory nerve.

Close to the sensory cells are the support cells, columnar in shape, with the nucleus located in the middle or apical region of the olfactory epithelium. Junction complexes and desmosomes are observed both between the cells of the basal region and between the cells of the apical region of the epithelium (Fig. 10). Desmosomes join support cells and sensory cells and support cells present microvilli on the surface. These microvilli project into the interior of the layer of mucous that covers the epithelium.

The non-sensory epithelium surface is irregular, with deep gaps and many mucous cells (Figs 13 and 14). Also, long superficial folds, and microvilli form a kind of labyrinth, and are generally associated with mucous cells (Fig. 13). Goblet cells with mucous secretion mostly occur in the free margins of the rosette. Goblet cells have a small basal region of cytoplasm with a nucleus, mitochondria, granular endoplasmic reticulum and a well-developed Golgi apparatus. Depending upon the phase of secretory activity, the appearance and the size of the goblet cells vary: when cells are larger mucous granules are nearer the surface (Fig. 14).

Mucous cells secrete neutral mucous as detected by the positive reaction to the PAS technique. Carboxylated and sulphated acid mucous, indicated by the positive reaction to Alcian...
Blue pH 2.5 and pH 1.0, respectively, were also found. The technique of Alcian Blue pH 2.5/PAS indicated that some cells possess both carboxylated acid mucous and neutral mucous.

**Ultrastructure of the lips**

The epidermis of both lips is broad with a variable number of cell layers, in which three layers stand out – basal, intermediate and surface (Fig. 15). The basal epithelial layer comprises cylindrical cells with oval to elongate nuclei, supported by a broad basal membrane (Figs 15 and 16). The intermediate region has elongated cells with the two extremities tapering off and forming a strongly united epithelium. Nearer the basal region, the nuclei are elongated and are more spherical closer to the apical region. All epithelium layers are strongly cohesive through a large quantity of desmosomes and hemidesmosomes, depending on the location (Fig. 17). Connective tissue is dense and made up of elastic and collagen fibers (Fig. 16). The apical layer is made up of squamous cells, with two to three layers, depending on the epithelial region. Viewed superficially, the cells are covered by short or punctiform microridges (Fig. 18).

Small numbers of mucous cells are on both lips, with the greatest concentration laterally. Mucous cells are globular and the nuclei are flat basolaterally. The mucous reacts positively to Alcian Blue pH 2.5 and to PAS and weakly to Alcian Blue pH 1.0, indicating neutral carboxylated mucous and a small quantity of sulphated mucous. These cells stand out from the epithelial cells from the inner layer because they become larger
Photoperiod and feeding behavior of the Antarctic fish *Notothenia rossii*

The upper lip has, in the central region, a slightly irregular surface with small protuberances and gaps. This surface becomes more irregular near the edges. Taste buds, of constant shape but variable size, are abundant in the central part of the lip (Figs 18-20). Taste buds have basal cells and support cells, with light nuclei in the middle. Sensory cells, with dark nuclei, are between support cells from which cytoplasmic processes extend towards the epithelial surface, where they become sensory (visible superficially, Fig. 20). Sensory cells are surrounded by undifferentiated epithelial cells covered by microridges. The taste buds of *Notothenia rossii* are elongated, between 100 and 130 µm in height. Many folds of microvilli are found on the apical pole of the taste bud.

**DISCUSSION**

*Notothenia rossii* is an excellent biological model for the study of sensory systems in teleost fish due to its ecological plasticity, wide geographical distribution ([Guin & Heemstra 1990](#)) and life cycle with its changes in the diet, distribution and growth ([Hureau 1970](#), [Tavernier 1972](#), [Burchett 1983a](#)). Morphological organization of sensory organs and their integration with fish response to sensory stimuli, are important for understanding the ecology and behavior of the species. More than one sensory system is probably always used by foraging fish to find food, hence it is difficult to generalize the relative importance of the roles of chemo and photoreception in the capture of prey. In aquatic systems smell and taste are carried...
in aqueous solutions. Smell is considered perception at a distance while taste requires contact of the element with the sensory structure (Hara 1971). Photoreception is also perception at a distance and depends on a complex retinal structure formed by photoreceptor cells, cones and rods.

When light was constant (photoperiod 24L), individuals were stimulated both chemically and visually, yet reactions were fewer and slower than during alternating periods of light and dark. In constant darkness with red light response to visual cues was least while response to chemical cues was similar to that of 12L/12D. Apparently in nature, N. rossi feeds less during winter than during summer, even when prey are abundant (Burchett 1983a). Nototheniids are slow-swimming carnivores in general (Ekaau 1991): for example, N. coriceps Richardson, 1844, is a sedentary species with limited mobility, at least at the end of spring and summer (Barrera-Oro & Cazaux 1990, Rios & Fanta 1998).

Notothenia rossii feeds opportunistically on prey when it is more abundant and more easily caught (Burchett 1983b). Morphological analysis of the sensory structures may demonstrate this capacity. Cone and rod photoreceptors along with taste buds on the upper and lower lips, and an olfactory rosette with a sensory and a non-sensory epithelium rich in mucous are characteristics that help these fish to detect and select food.

Photoperiods and the stimuli (chemical or visual) had similar response intervals. However, the shortest reaction time to the visual or chemical stimulus occurred with light, mainly in 12L/12D. Notothenia rossii reacts more readily to prey when stimulated visually. This may have been due to the methodology of constant motion of the piece of fish used as a stimulus during the entire experiment. Prey should be sufficiently mobile to stimulate fish (Duarte & Moreno 1981) and, in N. coriceps, pursuit of L. nudifrons began immediately once it began to swim away (Fanta 1999). In this context, perhaps an improvement on the experiment would be to include live bait, such as fish, krill or amphipods.

Sensory organs of N. rossii are complex when compared to some other Antarctic fish such as Gobionotothen gibberifrons Lønberg, 1905 or Ophthalmoptychus amberensis Tomo, Marshall & Torno, 1977, but similar to those of N. coriceps Richardson, 1844, Trematomus newnesi Boulenger, 1902, and Lepidonotothen nudifrons Lønberg, 1905 (Grooten & Fanta 1998, Meyer & Fanta 1998, Fanta et al. 1999, 2001, Donatti & Fanta 2001, 2002). With behavioral experiments and associated analysis of morphological structures permits a better understanding of feeding behaviors. Here we show that fish are more active foragers in light and under an alternating daylight regime.

ACKNOWLEDGEMENTS

We thank the CNPq (Projects 480.844/97-6 and 490.103/99-5) for financial support and for the support to E. Fanta (300.831/93-5); CAPES for a PICDT-Doctorate Scholarship to L. Donatti; the logistical support by the Secretariat of the Interministerial Commission for Marine Resources (SECIRM) and the staff of the Brazilian Antarctic Station Comandante Ferraz (EACF).

LITERATURE CITED


