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Aquaculture 263 (2007) 303–309
Aquaculture
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Olfactory and gustatory sensitivity to some feed-related chemicals in the Atlantic halibut (*Hippoglossus hippoglossus*)

S.Y. Yacoob *, H.I. Browman

Institute of Marine Research, Austevoll Research Station, N-5392 Storebø, Norway

Received 8 June 2006; received in revised form 1 November 2006; accepted 14 November 2006

Abstract

In farms, Atlantic halibut (*Hippoglossus hippoglossus*) exhibit low appetite to formulated feeds, resulting in slow growth rate and feed wastage. Addition of attractants and/or stimulants in feeds may motivate them to actively feed in optimum quantities. In this study, olfactory and gustatory (facial) nerve responses to some feed-related chemical compounds were recorded. The olfactory thresholds for 4 tested amino acids were: 10^{-7} M for alanine, 10^{-6} M for arginine and 10^{-4} M for methionine and glutamic acid; at 10^{-3} M, relative response magnitudes (expressed as a percentage of response to 10^{-3} M alanine) of 20 amino acids varied from 144% for methionine to 19% for aspartic acid. Gustatory nerve responses to 22 compounds including amino acids, bile acids, organic acids, and nucleotides were recorded. Only proline, adenosine monophosphate and inosine monophosphate elicited responses at 10^{-3} M; at 10^{-2} M, betaine and guanidine monophosphate also elicited large responses. The gustatory threshold for proline response was at 10^{-5} M. The results indicate that halibut possesses a generalized olfactory response pattern to amino acids that is comparable with other species and a very specific and narrow taste spectrum.

Keywords: Atlantic halibut; Olfaction; Gustation; Electrophysiology; Attractants; Stimulants

1. Introduction

Atlantic halibut (*Hippoglossus hippoglossus*, "halibut"), a large flatfish, is an important aquaculture species in several countries in the Northern Hemisphere. Formulated diets and feeding-culture techniques for on-growth of halibut are continuously being developed, with variable success. Halibut tend to stay on the bottom of culture tanks or sea-cages (Cordero et al., 1994), exhibiting low feeding motivation to formulated feeds (resulting in feed wastage) and concomitant slow growth rate (Tuene and Nortvedt, 1995; Kristiansen et al., 2004). Adding feeding attractants and/or stimulants to formulated feeds could ameliorate these problems, as demonstrated with other species (Jones, 1992; Takeda and Takii, 1992). Unfortunately, very little is known about smell and taste in halibut, reducing decisions about what compound(s) to add to formulated feeds to educated guesswork.

Wild halibut are found at depths between 50 and 2000 m. As adults, they are predominantly benthic, but also occasionally pelagic (Nielsen, 1986), feeding on fishes (cod, hake, pogge, sand-eels, herring, capelin), cephalopods, crustaceans and other bottom-dwelling animals (Nielsen, 1986; Bowman et al., 2000). The diel

* Corresponding author. Leigh Marine Laboratory, P.O. Box 369, Warkworth 0941, New Zealand. Tel.: +64 9 422 2611x83624; fax: +64 9 422 2613.
E-mail address: s.yahiya@auckland.ac.nz (S.Y. Yacoob).

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doi:10.1016/j.aquaculture.2006.11.005

activity and foraging patterns of halibut is not known, although some flatfish families are considered nocturnal (e.g. Soleidae). In their natural environment, a variety of prey-related chemical cues are available to the predators that trigger arousal, search and ingestion behavior. These cues are less prevalent in intensive aquaculture settings in which dry pellet diets are used.

The chemical cues detected by olfactory receptors are conveyed to the brain through the olfactory nerve (cranial nerve, CN I). Amino acids are a major class of olfactory stimuli that release food search behavior in a variety of fishes (Jones, 1992). The olfactory sensory cell density in Dover sole (*Solea solea*), which locates prey mostly by olfaction, is higher than in plaice (*Pleuronectes platessa*), which rely more on vision for feeding (Harvery, 1996). Nevitt (1991) reported that two flatfish species, rock sole (*Lepidopsetta bilineata*) and starry flounder (*Platichthys stellatus*), coughed (analogous to sniffing in higher vertebrates) at higher rates when presented with attractive odorants. Kaimmer (1998) studied the behavior of Pacific halibut (*Hippoglossus stenolepis*) towards hooks baited with fillets of chum salmon (*Onchorhyncus keta*) and observed that they oriented to bottom currents to locate the bait, possibly after initial arousal to the bait's odor. Although these studies demonstrate the central role of olfaction in flatfish feeding, data on the olfactory detection thresholds for amino acids and their relative stimulatory effectiveness in flatfishes are sparse.

Fish taste receptors are innervated by one of three cranial nerves: facial (CN VII), glossopharyngeal (CN IX) or vagus (CN X). The facial nerve innervates the anterior oral cavity and lips, and the other two cranial nerves innervate intra-oral taste buds. Facial and glossopharyngeal nerves have similar electrophysiological taste response characteristics (Kohbara and Caprio, 2001). Although taste sensitivity is species-specific, taste receptors generally respond to nutritionally relevant substances such as amino acids, quaternary ammonium compounds, nucleotides or nucleosides, organic acids and bile salts (Marui and Caprio, 1992; Carr et al., 1996). Taste is used by the animal mainly to determine the palatability of food, although in catfish (*Ictalurus* spp.) it is also used for chemotaxis (Bardach et al., 1967). Although many studies demonstrate that the addition of compounds such as L-amino acids, betaine and nucleotides and/or prey extracts, stimulate feed consumption in flatfishes (reviewed by Mackie et al., 1980; Reig et al., 2003), the taste physiology of flatfishes is little known.

Electrophysiological studies can provide basic information on the threshold and concentration–response characteristics of compounds stimulatory to the chemosensory organs. More elaborate neurological studies have identified receptor types and specificity (e.g. Nikonov and Caprio, 2004), mixture interactions (e.g. Derby, 2000), stimulus coding (e.g. Sato and Sorensen, 2003) and transduction mechanisms (e.g. Michel et al., 2003), mostly by recording electrical impulses from single nerve cells or nerve fibers. In combination, such studies can guide the development of formulated feeds with respect to which substances to include as attractants and stimulants (*sensu* Browman, 2005). Studies of this nature are rare, particularly in flatfishes (although see Velez et al., 2005).

The study presented here was undertaken to characterize the physiological sensitivity of smell and taste in halibut in order to identify possible feeding attractants–stimulants. This was accomplished by recording the neural responses from olfactory and facial (taste) nerves to putative (food-associated) chemical stimuli.

2. Materials and methods

2.1. Experimental animals

Cultured juvenile halibut (10.8±0.8 cm total length) were obtained from the Institute of Marine Research, Austevoll Research Station, Norway. These fish were cultured following standard practices (Mangor-Jensen et al., 1998), were reared in 1500 l tanks at 8–12 °C, and were fed with a commercial dry diet (*Marin* brand, EWOS, Bergen, Norway) using automatic feeders. The experiments complied with the Principles of Animal Care, publication no. 86-23, revised in 1985, of The National Institutes of Health and with the institutional animal care guidelines of the Institute of Marine Research, Norway.

2.2. Electrophysiological recordings

Each fish was initially anaesthetized lightly by immersion in seawater containing 50 mg/l MS-222, and then deeply by an intraperitoneal injection of 250 μl/100 g body weight Saffan™ (alphaxolone–alphadolone). With this dose, the animals remained anaesthetized throughout the experiment (typically 2 h). Anaesthetized fish were wrapped in a wet paper towel and secured in a cleft carved out of a sponge. The gills were irrigated through the mouth with filtered seawater (10 °C). Different individuals were used for olfactory and gustatory recordings.

Olfactory responses were recorded from the olfactory nerve (nerve twig recording, NTR) as described by Silver (1982). There are morphological and physiological differences between the upper and lower olfactory epithelia in flatfishes (Prasad Rao and Finger, 1984; Velez et al., 2005). Responses of upper epithelia were studied here. Halibut possess sessile olfactory bulbs with long olfactory nerves. The olfactory nerve runs halfway across the dorsal roof of the eye sockets before entering the neurocranium. Thus, deocculation of an eyeball readily exposed the anterior portion of the olfactory nerve. The nerve was cut centrally and dissected into several fine twigs. One of these twigs was placed on a pair of platinum–iridium hook electrodes and covered with liquid paraffin. The neural activity was AC-amplified (P55; Astro-Med, Inc., USA) under a band-pass filter setting of 30–3000 Hz. The amplifier output was monitored on an oscilloscope, digitized (National Instruments A/D board) and saved to PC using custom software (JASCO Research, Victoria, British Columbia, Canada). The multi-unit neural responses were integrated (time constant 0.5 s) using Chart software (AD Instruments, Australia) and the magnitude of the response to each stimulant was measured as the maximum height of the integrated trace.

Gustatory responses were recorded from a branch of the trigeminal (V)–facial (VII) nerve complex innervating the upper lip and the anterior part of the oral cavity. Typically, this complex runs ventrally through the eye-socket and is exposed by deocculation. In halibut, this nerve complex runs suborbitally and it was exposed by removing a piece of skin and the underlying tissue between the maxilla and the eye-orbit. Gustatory nerve preparation and recording of its neural activity was essentially the same as that for the olfactory nerve (both olfactory and gustatory responses were recorded from fine nerve twigs and their responses may be smaller than that of whole nerve bundles). The responsive area of the upper lip innervated by the twig being recorded was detected by tactile stimulation with a fine glass probe, as fish's taste buds are also sensitive to tactile stimuli.

2.3. Stimuli

Amino acids were used as olfactory stimuli. Amino acids, nucleotides, a bile acid and an organic acid were used as gustatory stimuli. These are the group of compounds known to mediate feeding behavior in fishes (Carr et al., 1996). Stock solutions of 10 mM amino acids were prepared weekly and stored at 4 °C until use. Nucleotide and bile acid solutions were prepared before each experiment. The method of stimulation is described in detail elsewhere (Yacoob et al., 2004). Briefly, the olfactory epithelium, or the taste receptor field was perfused with natural seawater at a constant rate of 6.0 ml/min and the stimulus (0.5 ml) prepared in the same water was added to this flow using a three-way solenoid valve, controlled by an electronic time switch. Blank seawater was used as a control. Each stimulant was tested at least twice (thrice in most cases) in the same preparation and they were presented in random order. After each stimulant, response to a standard solution (10^{-3} M alanine for olfactory recording and 10^{-3} M proline for taste recording) was tested to ensure that the preparation remained stable. Controls were undertaken in between stimulus presentations to eliminate the possibility of cross contamination. Relative stimulatory effectiveness (RSE; the magnitude of integrated responses expressed as a percentage of that of 10^{-3} M alanine or 10^{-3} M proline) of all test stimuli and threshold concentrations (concentration necessary to produce a response larger than that for the control) to some representative stimuli were determined.

3. Results

3.1. Olfactory nerve responses

Application of odorants to the olfactory epithelium elicited a large phasic response followed by a decline to a steady tonic level (Fig. 1a). The asynchronous nerve activity was integrated and the maximum height of the phasic response from the mean spontaneous level was considered to be the magnitude of the response. At suprathreshold concentrations, the response magnitude increased with the logarithmic increase in stimulus concentration (Fig. 1b). The thresholds were approximately 10^{-7} M for alanine, 10^{-6} M for arginine, and 10^{-4} M for methionine and glutamic acid (Fig. 1b). All amino acids tested elicited neural responses, and their RSE varied from about 19% for aspartic acid to 144% for methionine (Fig. 2).

3.2. Facial taste nerve responses

Typical taste responses of halibut are shown in Fig. 3a. Of 22 putative taste stimulants tested, only a small number of compounds were effective in halibut (Table 1). At 10^{-3} M, only proline and nucleotides elicited neural responses. At 10^{-2} M, glycine and glutamic acid elicited slight responses, whereas betaine evoked a large response. Gustatory responses to proline were of a fast-adapting phasic nature (Fig. 3a), whereas

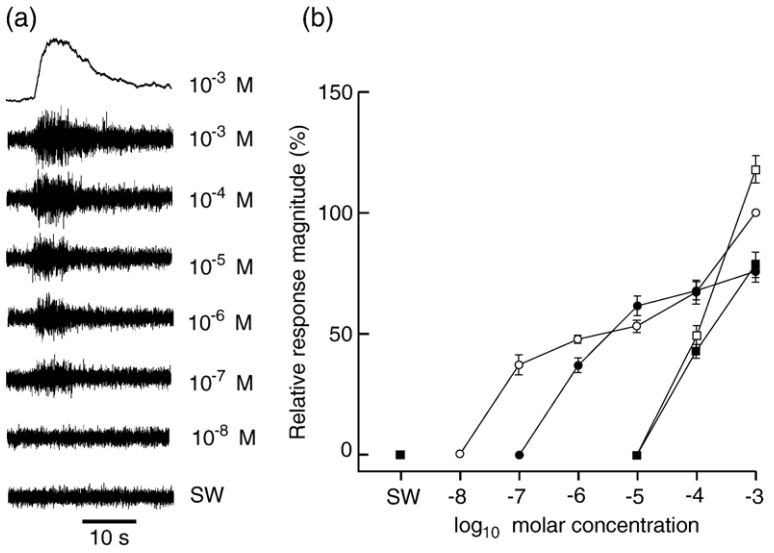

Fig. 1. Olfactory nerve responses recorded in juvenile Atlantic halibut. (a) Typical olfactory nerve responses, concentration–response series for alanine. The upper trace for 10^{-3} M is the integrated form of the lower; threshold is between 10^{-8} and 10^{-7} M. (b) Concentration–response curves for four amino acids: methionine (□), glutamic acid (■), alanine (○) and arginine (●). The response magnitudes are expressed as percentage of responses to 10^{-3} M alanine. Bars are standard errors, $n=4$.

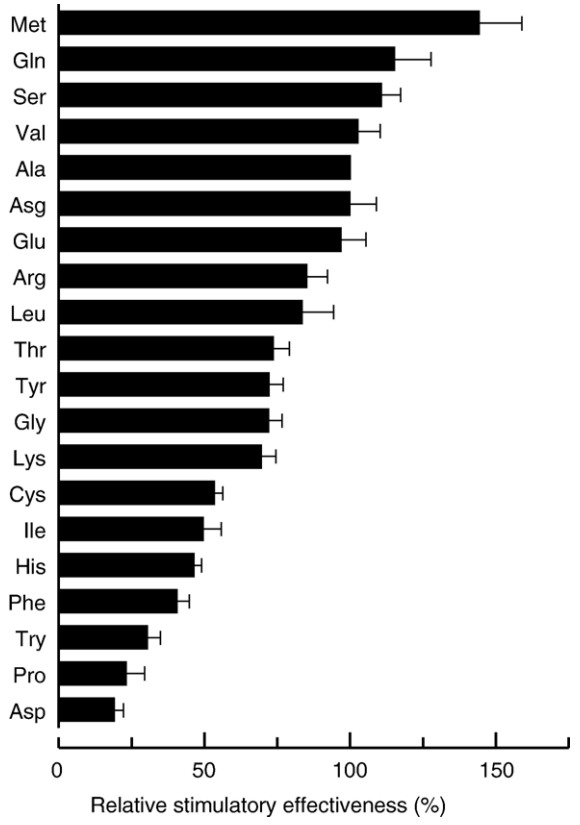

Fig. 2. Relative stimulatory effectiveness of amino acids at 10^{-3} M in eliciting olfactory nerve responses in juvenile halibut. The values are expressed as a percentage of the responses to 10^{-3} M alanine. Bars are standard errors, $n=5$.

tonic components of nucleotide responses were longer than that of proline. The threshold response for proline was approximately 10^{-5} M (Fig. 3a). Thresholds for nucleotides and betaine were approximately 10^{-3} and 10^{-2} M, respectively, and their concentration–response curves were relatively steep (Fig. 3b and Table 1). Guanosine monophosphate elicited only a slight response at 10^{-3} M, but at 10^{-2} M it was more than five times as sensitive as 10^{-3} M proline.

4. Discussion

Exposing the olfactory nerve by removing the cranium between the eyes is difficult in flatfishes (see Velez et al., 2005). The eye-socket approach reported here provides clear access to it. In this study, electrical responses were recorded from multiple nerve fibers and quantified as the peak height of the integrated form of the nerve activity. The sensitivity of this type of recording may be slightly less than recording from a single cell or fiber. However, it accurately characterizes the overall sensitivity of the sensory organs and has been used in a large number of studies (reviewed by Hara, 1992).

The food search behavior of halibut in the wild is unknown. However, the fact that they are traditionally caught using baited longlines (Kenchington, 1996) indicates that they respond to prey odors. The current study provides a physiological basis for the olfactory

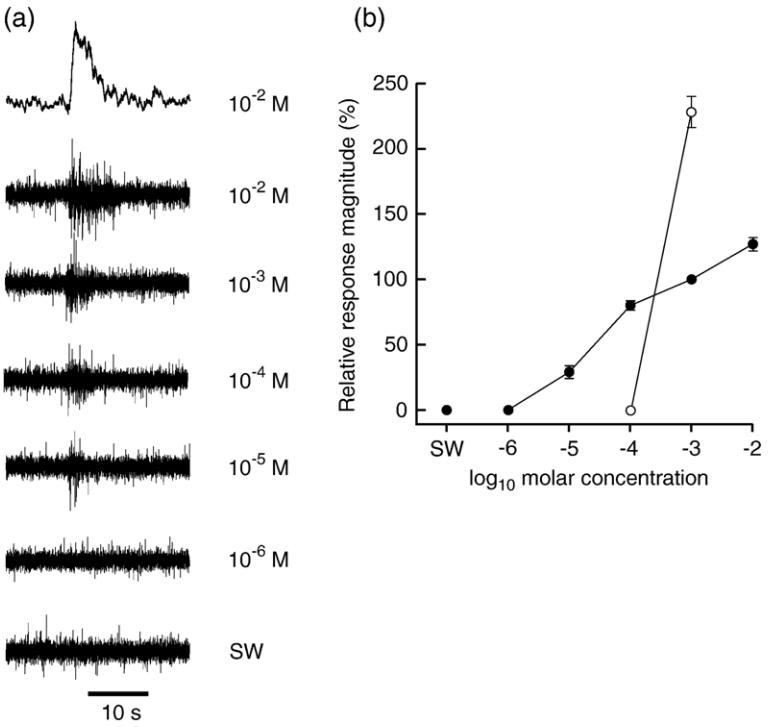

Fig. 3. Facial taste nerve responses recorded in juvenile Atlantic halibut. (a) Typical gustatory nerve responses, concentration–response series for proline. The upper trace for 10^{-2} M is the integrated form of the lower; threshold is between 10^{-6} M and 10^{-5} M. (b) Concentration–response curves for proline (●) and inosine monophosphate (○). The response magnitudes are expressed as percentage of responses to 10^{-3} M proline. Bars are standard errors, $n=4$.

search potential in halibut. The electrical characteristics of the olfactory nerve, threshold response levels, concentration–response characteristics, and spectrum of stimulatory amino acids reported here for halibut are comparable to that for other fishes (Hara, 1992). Although olfactory response patterns are similar across the fish species studied to date, there is some species specificity in responses to specific amino acids (discussed in Yacoob et al., 2004). The only other flatfish for which olfactory data is available is Senegalese sole (*Solea senegalensis*; Velez et al., 2005). The ranking of normalized electro-olfactogram amplitudes at 10^{-3} M of amino acids on the upper nostril of the Senegalese sole (calculated from the data presented in the figures) was: methionine > alanine > glutamine > cysteine > arginine > leucine > lysine > serine > threonine > glycine > asparagine > tryptophan > isoleucine > tyrosine > phenylalanine. This ranking is similar to that for halibut (Fig. 2). However, response to cysteine and asparagine is 62% and 104% of that for alanine in halibut, while it is about 91% and 47% in sole. It is not clear if these differences are species specific or simply due to different recording methods.

In this study, taste responses to a representative subset of known fish feeding stimuli were tested by recording from the facial nerve. Of 22 compounds applied to the taste receptors, only a small number elicited neural responses (Table 1). The taste responses of some fish are limited to only to few compounds (e.g. rainbow trout, *Oncorhynchus mykiss*; Kohbara and Caprio, 2001); the so-called 'limited response group'. However, halibut appears to have the narrowest taste spectrum of any teleost tested to date. The ability to taste a wide variety of compounds depends on the number of receptor types available, which seems to be either evolved or regressed phylogenetically (Hara et al., 1994; Finger, 1997). The receptors for proline are present in all fish species studied so far (reviewed by Marui and Caprio, 1992). Proline receptors are also present in halibut: in fact, it is the only amino acid receptor. Nucleotides and betaine are other important classes of taste stimuli for halibut, but only at high concentrations, which is not surprising because these compounds might be released directly inside the mouth when the prey is bitten.

Feeding stimulants for flatfishes have been identified by behavioral observation and/or feeding trials: IMP and

Table 1
Relative stimulatory effectiveness (RSE) of chemical stimuli at 10^{-3} and 10^{-2} M in eliciting taste responses in the facial nerve of the Atlantic halibut

Stimuli	10^{-3} M	10^{-2} M
Proline	100	127.0±5.8
Aspartic acid	0	0
Tryptophan	0	0
Phenylalanine	0	0
Lysine	0	0
Glycine	0	19.5±7.8
Tyrosine	0	0
Glutamic acid	0	27.9±9.4
Asparagine	0	0
Alanine	0	0
Valine	0	0
Serine	0	0
Glutamine	0	0
Methionine	0	0
Betaine	0	164.9±26.9
Taurine	0	0
Inosine	0	0
Taurolithocholic acid	0	0
Lactic acid	0	0
Adenosine monophosphate	80.0±30.7	–
Inosine monophosphate	218.3±26.2	–
Guanosine monophosphate	14.0±4.1	513.3±30.6

The values are expressed as a percentage (mean±S.D.; $n=5$) of the responses to 10^{-3} M proline; dashes refer not tested.

inosine for turbot (Mackie and Adron, 1978), inosine, glycine–betaine, betaine, trimethylglycine, glycine, alanine, and glutamic acid for Dover sole (Mackie et al., 1980; Metailler et al., 1983; Reig et al., 2003). This narrow spectrum of stimulants is consistent with the taste nerve responses reported here. However, it is interesting to note that glycine and glutamic acid produced only weak taste responses even at 10^{-2} M while alanine produced none at all (Table 1). Glycine and alanine are the two most frequently cited stimulants in about 35 fish species (Carr et al., 1996). The compounds that flatfishes respond to are major tissue metabolites of molluscs and crustaceans rather than fish (see Carr et al., 1996). Indeed, crustaceans typically constitute a large percentage of the diet in halibut (Bowman et al., 2000). However, the same study showed that fish is also a dominant prey item (up to 98% in some tested categories) for halibut. Nonetheless, the major fish tissue metabolites (e.g. lactic acid, taurine and histidine) did not elicit any taste response (Table 1).

Halibut possesses a generalized olfactory response pattern for amino acids and a very specific and narrow taste spectrum, which matches to that of molluscan and crustacean tissue metabolites. Behavioral studies to identify the sequence and/or combination of sensory modalities (vision, olfaction, taste and mechanosensation), and the specific chemical compounds involved in the search and ingestion phases of halibut feeding, would be useful in developing formulated feeds for this species. Nucleotide and quaternary ammonium compound fractions of crustacean/molluscan extract appear to be the promising source of *stimulants*, while amino acid fractions for *attractants*.

Acknowledgments

This research was supported by the Research Council of Norway (Project No. 146750/120) and by the Institute of Marine Research, Norway. We thank Per Anders Jensen and Jan Erik Fosseidengen for assisting in the experiments, and Sjur Åge Skår for the supply of halibut juveniles.

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