

Short communication

Prey extracts evoke swimming behavior in juvenile Atlantic halibut (*Hippoglossus hippoglossus*)

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Abstract

This study tested the efficacy of prey extracts to induce food search behavior in juvenile (15–25 cm total length, 0 year group) Atlantic halibut (*Hippoglossus hippoglossus*). In square culture tanks, halibut responded to shrimp and squid extract by tightly turning towards the source of the stimulus and by swimming in circles for 2–5 min following stimulus delivery. Cod extract, or a synthetic mixture consisting of glycine, proline, betaine, arginine, and alanine, failed to evoke this behavior. © 2007 Elsevier B.V. All rights reserved.

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1. Introduction

Metabolites associated with food, such as free amino acids, quaternary ammonium compounds, nucleosides or nucleotides and organic acids evoke feeding behavior in predators (see Carr et al., 1996). It is also possible that there may be other stimulatory compounds present in prey exudates that also motivate feeding responses (e.g. lecithin, Harada, 1987). Thus, whole prey extracts are a promising source of compounds that might serve to stimulate feeding and uptake of commercial diets for fishes (e.g. Kolkovski et al., 1997). Behavioral responses to whole prey extracts can also be used as a reference against which the potency of its components can be tested.

In fishes, olfaction appears to mediate arousal/search for food while gustation is involved in the decision to ingest (reviewed by Finger, 1997). In flatfish, some of the responses elicited in response to the presentation of chemical compounds (odors) are: larval feeding behavior (Knutzen, 1992), weaning (Metailler et al., 1983), stimulation of feeding (Mackie and Mitchell, 1985; Reig et al., 2003), motivated responses to hooks baited with fish (Kaimmer, 1998) and active olfactory sampling ('sniffing', Nevitt, 1991). Interspecific variation in the relative roles of olfaction and vision in feeding have also been assessed: plaice (*Pleuronectes platessa*) depend more on vision, but sole (*Solea solea*) relied principally on chemoreception and mechanoreception (Batty and Hoytt, 1995).

Atlantic halibut (*Hippoglossus hippoglossus*, "halibut") is a high-value flatfish, reared intensively using fishmeal-based diets. Halibut lie inactive at the tank bottom and exhibit a poorly motivated feeding response to formulated diets (Tuene and Nortvedt, 1995; Kristiansen et al., 2004). Chemical stimulants that

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could motivate a stronger feeding response would, therefore, be useful in developing an attractive diet. However, the behavioral response of halibut to chemical feeding stimuli is unknown. In a recent electrophysiological study (Yacoob and Browman, 2007), we established the relative stimulatory effectiveness of typical feeding stimulants to the olfactory and gustatory organs in halibut. Here, we report upon the results of a follow-up study in which the food search behavior of halibut in response to prey extracts was evaluated.

2. Materials and methods

2.1. Experimental animals

Cultured juvenile halibut (total length \pm SD, 21.1 ± 2.1 cm; 0 year group; sex not determined) 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. Experimental fish were fed to satiation, with the same diet, once a day in the evening. 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. Experimental tanks

Experiments were conducted in tanks similar to rearing units. These are fiberglass, flow-through, square tanks with rounded corners (100 \times 100 \times 165 cm; length \times width \times height, water depth 100 cm, volume 1000 l). Filtered seawater (8–10 °C) was circulated through the tanks at the rate of 10 l min⁻¹ and drained centrally, yielding an exchange rate of 60% every hour. Experimental halibut were acclimated for at least 2 d before the tests began. Individuals that swam vertically at the surface, an abnormal behavior (Kristiansen et al., 2004), were replaced with normal ones.

2.3. Stimuli and stimulation method

Behavioral responses of halibut to the extracts of red shrimp (*Pandalus borealis*), squid (*Loligo* sp.) and cod (*Gadus morhua*) were observed. These represent the major phyla of prey for Atlantic halibut (Tables B-58 a and b in Bowman et al., 2000). Extracts were prepared as follows: 50 g of muscle from shrimp, cod or the

mantle from squid were homogenized in a blender for 2 min in 300 ml cold seawater. The homogenate was centrifuged at 5000 rpm for 15 min at 4 °C. The supernatant was filtered and frozen as 25 ml aliquots at -30 °C until used (a maximum of 7 d). Stimulus solutions were prepared by mixing 10–30 ml (see Results for the volumes used) of extracts in 100 ml of seawater collected from the experimental tanks just prior to stimulation. In addition to the extracts, response to a synthetic mixture of 0.49 mM proline, 0.25 mM alanine, 0.20 mM arginine, 1.59 mM glycine and 0.38 mM betaine was also tested. These five compounds are ubiquitous in crustaceans and cephalopods and stimulate feeding in about 35 species of fish (see Carr et al., 1996). The concentrations of compounds used in the mix were equimolar of that present in 10 ml of shrimp extract; this is a potent dose of odorant (see Results).

Stimuli (100 ml) were gravity-fed into the bottom of the tank through a 3 mm Teflon tube at a rate of 5 ml s⁻¹. Dye tests demonstrated that the odor solutions spread throughout the entire tank in ca. 70 s. To avoid giving fish a hydrodynamic cue associated with stimulus flow, seawater flowed continuously through the stimulus delivery tube. This flushing also eliminated the possibility of cross-contamination of stimulants. The stimuli and seawater were switched using a manual three-way Teflon valve. The tank was surrounded by a black drape to avoid triggering escape behaviors due to movement around the testing area.

2.4. Test protocol

Five replicates, each including 25 acclimated individuals, were run. In each replicate, the following six treatments were tested: normal (before stimulation), seawater from experimental tank (control), red shrimp extract, squid extract, cod extract and synthetic mix. Each treatment was tested at least 3 times in the same replicate and means were used in statistical analysis (ANOVA and *post-hoc* Holm–Sidak). The odorants (treatments) were presented once a day between 10:00–11:00 h and the behavior of halibut was videotaped from above using a custom-built camera connected to a Sony Video-walkman (GV-S50 E). In each stimulation event, behavior was recorded for a total of 9 min: 3 min with only background seawater flowing into the test tank (normal), 3 min after control was presented through the odor delivery tube and 3 min after the test odor presentation. Behavioral changes observed between 70 and 130 s after stimulation with extracts of shrimp, squid, cod and synthetic mix were compared with that of the control.

3. Results

Halibut normally lay motionlessly in clusters, with only a few individuals swimming along the bottom and a few others occasionally relocating themselves on the tank bottom. Responses to attractive odors consisted of the fish turning tightly towards the direction of the odor stream and swimming around the tank for 2–5 min. This behavior was consistent and predictable in every stimulation event and in all replicates. Consequently, the number of halibut that swam for a minimum of 1 min in response to odorant (counted from video playback) was taken as a measure of the stimulatory effect of odorants. In some instances, odor stimulation also initiated agonistic behavior such as nipping and chasing of neighbours. Initial tests showed that 10.0 ml (equivalent of extract from 1.7 g wet weight of tissue) of red shrimp extract was adequate to induce a predictable response. Subsequently, 10.0 ml of each prey extract was tested. If this concentration failed to induce a response, higher concentrations (up to 30 ml) were used.

There was a significant odor stimulation effect ($F(4, 20)=69.239$, $P<0.001$). Under normal conditions, only 13% of halibut swam actively. Presentation of control caused no significant changes in the number of fish swimming (*post-hoc* Holm–Sidak test, $P=0.75$, Fig. 1). However, when stimulated with shrimp and squid extracts, 85% and 59% of fish displayed swimming behavior respectively, which was significantly higher than the response to control ($P<0.001$; Fig. 1). Response to cod extract and synthetic mix was not significantly different from the control ($P=0.41$ and 0.11 respectively; Fig. 1). Increasing the concentrations of these two odors to

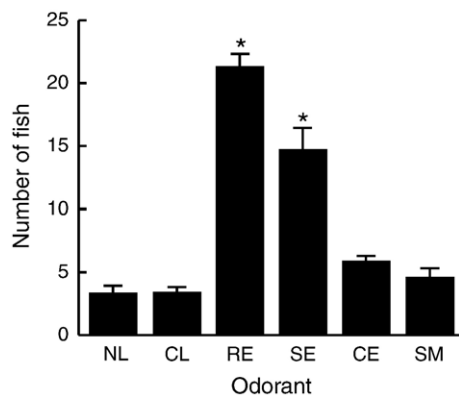


Fig. 1. Mean number of halibuts out of 25 (+SE; $n=5$) swimming before and after odor stimulation. NL: normal (before), CL: seawater (control), RE: red shrimp extract, SE: squid extract, CE: cod extract, and SM: synthetic mixture (see text for composition and concentrations). The asterisk refers to significant differences (see text).

even 3 times the effective shrimp extract concentration failed to induce swimming behavior.

4. Discussion

Extracts of red shrimp or squid, but not cod, were potent feeding attractants for halibut. Since only halibut larger than those used in this study will likely feed on cod in the wild, it is possible that a response to cod extract would have been manifested in larger fish. However, it is more likely that the attractiveness of a prey item to halibut is dependent upon its constituents.

Crustaceans and cephalopods, but not fish, contain high concentrations of low molecular weight organic solutes, notably alanine, glycine, arginine, proline and betaine, which all stimulate feeding in fishes (Carr et al., 1996). Interestingly, a mixture of these 5 compounds alone failed to induce swimming behavior in halibut. Therefore, some minor constituents in the shrimp and squid extracts must have contributed to their stimulatory effectiveness. For example, tryptophan is a minor constituent of krill extract and it was the only one of 20 amino acids in the extract that was a feeding stimulant for jack mackerel, *Trachurus japonicus* (Ikeda et al., 1988). Alternatively, a single compound at high concentration might also be effective, as observed in winter flounder (*Pseudopleuronectes americanus*) that were attracted by glycine (Sutterlin, 1975).

The presence of prey typically triggers an intensive area restricted food search behavior in fishes, characterized by an increase in turning per unit distance (klinokinetic response), low speed and short move lengths (Benhamou and Bovet, 1989). The number of turns $>90^\circ$ undertaken by brown bullhead catfish (*Ameiurus nebulosus*) has been used to quantify its behavioral (olfactory) responsiveness to amino acids (Valentincic et al., 2000). In the laboratory, juvenile plaice (*P. platessa*) displayed a klinokinetic response to the presence of buried bivalve prey, although it is not known whether the cue triggering this behavior was visual, tactile or chemical (Hill et al., 2000).

Odor stimulation evoked swimming behavior in halibut. Agonistic interactions, such as chasing and nipping, were also manifested. The swimming response was characterized by rapid turning toward the odor source and initiation of steady swimming around the tank, without frequent turning. This typical and highly reproducible behavior could be developed into an assay to identify chemical attractants for flatfish.

Generally, it is olfactory detection of prey odors that evokes arousal in fishes and guides them in swimming towards prey (chemotaxis). Taste is used mainly to

determine the palatability of food, although in catfish (*Ictalurus* spp.) it is also used for chemotaxis (Bardach et al., 1967). Our earlier electrophysiological study (Yacoob and Browman, 2007) indicated that the olfactory sensitivity of halibut extended across a wide range of amino acids but that gustatory sensitivity was far more limited: of 22 taste stimulants tested, only proline, monophosphates of adenosine, inosine and guanidine, and betaine evoked gustatory response. The relative contribution of olfactory and gustatory senses in mediating the swimming behavior reported here requires further investigation.

The information reported here is a necessary first step in identifying feeding attractants for use in the development of a formulated dry feed that will evoke feeding responses in halibut. In order to be used with off-the-shelf convenience, and without sanitary concerns, the active compounds in the extracts must be identified and an effective mode of stimulant delivery (i.e. adding to the tank water during feeding or incorporation in the feed itself) must be developed. The potential use of attractants in weaning diets for halibut larvae should also be evaluated. The large quantities of blanch waters discarded from shrimp and squid-processing plants is a potential source of attractants.

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