Taste preferences in fish

Alexander O Kasumyan[1] *& Kjell B Døving*[2]

[1]Department of Ichthyology, Faculty of Biology, Moscow State University, 119992 Moscow, Russia; [2]Department of Biology, University of Oslo, N-0136 Oslo, Norway

Abstract

The fish gustatory system provides the final sensory evaluation in the feeding process. Unlike other vertebrates, the gustatory system in fish may be divided into two distinct subsystems, oral and extraoral, both of them mediating behavioural responses to food items brought in contact with the fish. The abundance of taste buds is another peculiarity of the fish gustatory system. For many years, morphological and electrophysiological techniques dominated the studies of the fish gustatory system, and systematic investigations of fish taste preferences have only been performed during the last 10 years. In the present review, basic principles in the taste preferences of fish are formulated. Categories or types of taste substances are defined in accordance with their effects on fish feeding behaviour and further mediation by the oral or extraoral taste systems (incitants, suppressants, stimulants, deterrents, enhancers and indifferent substances). Information on taste preferences to different types of substances including classical taste substances, free amino acids, betaine, nucleotides, nucleosides, amines, sugars and other hydrocarbons, organic acids, alcohols and aldehydes, and their mixtures, is summarised. The threshold concentrations for taste substances are discussed, and the relationship between fish taste preferences with fish systematic position and fish ecology is evaluated. Fish taste preferences are highly species-specific, and the differences among fish species are apparent when comparing the width and composition of spectra for both the stimulants and the deterrents. What is evident is that there is a strong similarity in the taste preferences between geographically isolated fish populations of the same species, and that taste preferences are similar in males and females, although at the individual level, it may vary dramatically among conspecifics. What is noteworthy is that taste responses are more stable and invariable for highly palatable substances than for substances with a low level of palatability. Taste preferences as a function of pH is analysed. There is a good correspondence between development of the gustatory system in fish ontogeny and its ability to discriminate taste properties of food items. There is also a correspondence between oral and extraoral taste preferences for a given species; however, there is no correlation between smell and taste preferences. Taste preferences in fish show low plasticity (in relation to the diet), appear to be determined genetically and seem to be patroclinous. Fish feeding motivation and various environmental factors like water temperature and pollutants such as heavy metals and low pH water may shift fish taste preferences. Comparisons between bioassay and electrophysiological data show that palatability is not synonymous with excitability in the gustatory system. The chemical nature of stimulants and deterrents in various hydrobionts is outlined. The significance of basic knowledge in fish taste preferences for aquaculture and fisheries is emphasised.

Key words feeding behaviour, fish, food intake, gustatory system, taste preferences, taste stimulants

Correspondence:
Alexander O Kasumyan,
Department of Ichthyology, Faculty of Biology, Moscow State University,
119992 Moscow,
Russia
E-mail:
alex.kasumyan@mail.ru

Received 17 July 2002
Accepted 3 April 2003

© 2003 Blackwell Publishing Ltd

 © 2003 Blackwell Publishing Ltd,

Introduction

The aim of the present review is to provide a basis for understanding fish feeding behaviour through an examination of the taste preference fish have for different foods and the single chemical components of the food.

Feeding in fish, as in other groups of animals, is an important function of life, and is a result of processes that are associated with searching, aiming, accepting, seizing, oral processing and evaluation of the quality of food objects. Swallowing, digestion, absorption and assimilation follow these processes. The success of these tasks, directly related to the satisfaction of energy requirements, is responsible for the rate of growth, maturation and fecundity of fish, their social status, migratory activity, life strategy and their resistance to the impact of unfavourable factors.

Many sensory systems contribute to fish feeding behaviour, but their role and significance may profoundly differ at different phases of the feeding behaviour. The consummatory phase of the feeding behaviour starts with the awareness of a food object and terminates with either swallowing or rejection. The various sensory cues involved in these tasks vary, but what determines the palatability (taste) of a food object for the fish involves predominantly gustatory and some aesthetic qualities. Various mechanosensory organs, and what is termed as a common chemical sense, can mediate these latter qualities. In many instances, a fish rejects a food item after it has been taken into the mouth cavity (Bardach *et al.* 1959; Tester 1963; Atema 1980; Gerhart *et al.* 1991; Schulte and Bakus 1992). Such behaviour demonstrates that an object that is selected as a food item, based on the information given by any of the sensory systems available, is not sufficient to guarantee its suitability as food for the fish. The sensory systems in question are olfaction, vision, acoustic, lateral line organ, extraoral gustatory subsystem or electroreception. This consideration indicates the important control functions of receptors located within the fish's mouth cavity in evaluating the specific food items.

The gustatory sensory system provides the final evaluation in the feeding process. It is well known from both scientific research and the anecdotes of fishermen that the taste properties of feeds or baits have a dramatic influence on food consumption of fish, growth rate and fishing success (Jones 1980; Mackie and Mitchell 1985; Takeda and Takii 1992; Kasumyan 1997). However, other sensory systems also participate in the consummatory phase in fish. The sense of touch or mechanoreception is functionally and structurally connected to the gustatory system (Kanwal and Caprio 1988; Hayama and Caprio 1989, 1990). It has been noted, for example, that an increased hardness or presence of acute outgrowths and spines on the external integument of the food organisms results in a decrease in consumption of such organisms by fish (Hunter 1980; Lemm 1983; Stradmeyer *et al.* 1988; Stradmeyer 1989). The number of such examples is limited, and thus may indicate an auxiliary role of mechanoreception in fish feeding behaviour. Additionally, althougth there are numerous solitary chemosensory cells covering the oral cavity of fish (Whitear 1992; Whitear and Moate 1994), there are few data on the functional properties of this common chemical sense (Silver 1987; Silver and Finger 1991; Kotrschal 1996), and we do not know what role this chemosensory system plays in the oral evaluation of prey quality. Furthermore, there are no data to indicate an obvious role of the common chemical sense in the final phase of fish feeding behaviour (Kotrschal 1991, 1992, 1995; Whitear 1992).

Studies of the gustatory system of fish began in the 19th century, and for many years, morphological techniques dominated this area of research. In the last decades, electro-physiological methods have come into use to evaluate the properties of the gustatory system. Presently, there are several reviews on the fish gustatory system, focusing on fish taste bud morphology and distribution (Kapoor *et al.* 1975; Tucker 1983; Jakubowski and Whitear 1990; Gomahr *et al.* 1992; Reutter 1992; Reutter and Witt 1993), the physiology of the peripheral taste neurones (Bardach and Atema 1971; Caprio 1982; Tucker 1983; Caprio 1984; Caprio 1988; Hara 1994a,b), the molecular mechanisms of taste receptor transduction (Brand and Bruch 1992) and the organisation of gustatory neural pathways within the central nervous system of fish (Kanwal and Finger 1992).

Methods for estimation of fish taste preferences have been nonexistent or at best, primitive, and have not been elaborated until recent times. When studying taste preferences in fish, it is difficult to avoid the influence of other chemosensory systems and, while it is easy to deprive a fish of the olfactory system, it is unrealistic to remove the taste organs or impede taste perception. Thus, many of the investigations developed with the purpose of studying taste preferences yielded results which reflected not only the gustatory system, but also the olfactory system, which is another principal chemosensory system

that mediates important cues and induces fish feeding behaviour (Atema 1980). The results of these studies as a basis for evidence of fish taste preferences should be used with significant caveats, apprehensions and restrictions. A decade ago, there were only limited data focusing on the taste preferences of fish; however, systematic investigations of fish taste preferences have been performed during the last 10 years (Kasumyan 1997).

In the present review, we summarise the appropriate information on taste preferences in fish, include unpublished data and use the information to formulate basic principles in the taste preferences of fish. We shall also evaluate the relationship between fish taste preferences with fish systematic position and fish ecology. Interesting aspects of the taste preferences in fish are variations associated with age and population and feeding experience in early life. The individual variability within a species is another aspect that will be considered, as will the effects of various biotic and abiotic factors on taste preferences. There is a large number of species mentioned in this review; therefore, we have listed the species with Latin and family names in Table 1. The prey of some fish is listed in Table 2.

The gustatory system in fish

The peripheral organs belonging to the gustatory system are the taste buds. Taste buds constitute the structural basis of the gustatory system in all gnathostomes – fish, amphibians, reptiles, birds and mammals. Jawless fish (hagfish (Myxinidae) and lamprey (Petromyzontidae) have end buds – organs that resemble the teleost taste buds (Reutter 1986). Phylogenetically, it seems likely that the taste buds were not consecutively and progressively developed from one vertebrate class to the next (Reutter and Witt 1993). The first descriptions of fish taste buds were published early in the 19th century (Weber 1827; Leydig 1851). Since these times, numerous articles concerned with taste bud morphology in various fish species have appeared (for reviews, see Reutter (1986) and Jakubowski and Whitear 1990).

In fish, the taste buds are situated not only within the oral cavity, pharynx, oesophagus and gills, but may also occur on the lips, barbels and fins, and over the entire body surface in many species. These peculiarities distinguish fish from other groups of gnathostomes, except the amphibians, as in some developmental stages, the taste buds are found in amphibians within the oral cavity and also in the skin of the head. The oral taste buds have an endoderm origin (Barlow and Northcutt 1995), while the external taste buds are claimed to be of ectodermal origin (Kapoor *et al.* 1975). The abundance of taste buds is another peculiarity of the fish gustatory system: taste buds are more numerous in fish than in any other animal. For instance, channel catfish, 35–39.5 cm in body length, have 680 000 $\pm$ 36 000 taste buds on the entire body and fins surface (Finger *et al.* 1991), which is nearly 100 times more than is found in the oral cavity of an adult man. The taste bud density varies according to the location and fish species. In the gular region of some benthivorous cyprinids, the density of the external taste buds reaches up to 300 mm^{-2}, but is much lower in other areas of the body surface and fins. The density of external taste buds is lower in planktivorous and surface-feeding cyprinids than in the bottom feeders (Gomahr *et al.* 1992). In cyprinids, the density of oral taste buds is between 300 and 400 mm^{-2} in the palatal organ (Osse *et al.* 1997) and 30–35 mm^{-2} in the area surrounding the teeth of salmonids (Marui *et al.* 1983a; Hara *et al.* 1993).

A typical taste bud has an ovoid or pear shape; its long axis is orientated vertically to the apical surface of the epithelium, which is normally not keratinised. As a rule, the base of a taste bud is situated on top of a small ascending papilla of the corium (dermis). Consequently, a taste bud is often situated on a dome on the epidermis (type I); in other cases, taste buds are slightly elevated (type II; Fig. 1) or are not elevated (sunken; type III; Reutter *et al.* 1974). Marginal cells form the border or margin between a taste bud and the neighbouring stratified squamous epithelium. Oral and extraoral taste buds consist of gustatory receptor, supporting and basal cells. Both gustatory receptor cells and supporting cells have an elongated shape, run more or less parallel and follow the long axis of the taste bud. They reach the surface of the epithelium by a pore (Fig. 1), which has a diameter of 2–5 μm to more than 20 μm (Jakubowski and Whitear 1990; Reutter 1992). The number of gustatory cells in a taste bud varies from five, as in the goby (*Pomatoschistus* sp.), to 67, as in the armoured catfish (Jakubowski and Whitear 1990). Gustatory receptor cells terminate with large receptor microvilli, which are conical in shape and measure up to 2.5 μm in length and about 0.3 μm in width. These cells form the receptor area or receptor field in the pore. An apex of supporting cells bears up to 10 small cylindrically shaped microvilli, which are about 0.5 μm long and about 0.1 μm wide. The supporting cells

© 2003 Blackwell Publishing Ltd,

Table 1 The index of common and scientific names for fish species

Common name	Latin name	Family, order
Alluaudi cichlid	*Haplochromis (Astatoreochromis) alluaudi*	Cichlidae, Perciformes
Angelfish	*Pomacanthus* spp.	Pomacanthidae, Perciformes
Angelfish	*Holacanthus* spp.	Pomacentridae, Perciformes
Anglerfish	*Lophius piscatorius*	Lophiidae, Lophiiformes
Arctic flounder	*Liopsetta glacialis*	Pleuronectidae, Pleuronectiformes
Armoured catfish	*Corydoras* sp.	Callichthydae, Siluriformes
Atlantic cod	*Gadus morhua*	Gadidae, Gadiformes
Atlantic herring	*Clupea harengus harengus*	Clupeidae, Clupeiformes
Atlantic salmon	*Salmo salar*	Salmonidae, Salmoniformes
Bitterling	*Rhodeus sericeus amarus*	Cyprinidae, Cypriniformes
Black molly	*Poecilia sphenops*	Poeciliidae, Cyprinidontiformes
Blue-green chromis	*Chromis viridis*	Pomacentridae, Perciformes
Bluehead wrasse	*Thalassoma bifasciatum*	Labridae, Perciformes
Bream	*Abramis brama*	Gadidae, Gadiformes
Brill	*Scophthalmus rhombus*	Scophthalmidae, Pleuronectiformes
Brook char	*Salvelinus fontinalis*	Salmonidae, Salmoniformes
Brown trout	*Salmo trutta*	Salmonidae, Salmoniformes
Bullhead	*Ictalurus natalis*	Ictaluridae, Siluriformes
Capelin	*Mallotus villosus*	Osmeridae, Salmoniformes
Channel catfish	*Ictalurus punctatus*	Ictaluridae, Siluriformes
Chinook salmon	*Oncorhynchus tshawytscha*	Salmonidae, Salmoniformes
Chub	*Leuciscus cephalus*	Cyprinidae, Cypriniformes
Chum salmon	*Oncorhynchus keta*	Salmonidae, Salmoniformes
Common carp	*Cyprinus carpio*	Cyprinidae, Cypriniformes
Crucian carp	*Carassius carassius*	Cyprinidae, Cypriniformes
Dab	*Limanda limanda*	Pleuronectidae, Pleuronectiformes
Dace	*Leuciscus leuciscus*	Cyprinidae, Cypriniformes
Dover sole	*Solea solea*	Soleidae, Pleuronectiformes
Dusky grouper	*Epinephelus guaza*	Serranidae, Perciformes
European eel	*Anguilla anguilla*	Anguillidae, Anguilliformes
European grayling	*Thymallus thymallus*	Salmonidae, Salmoniformes
European minnow	*Phoxinus phoxinus*	Cyprinidae, Cypriniformes
European sea bass	*Dicentrarchus labrax*	Moronidae, Perciformes
Five-bearded rockling	*Ciliata mustela*	Phycidae, Gadiformes
Flathead grey mullet	*Mugil cephalus*	Mugilidae, Mugiliformes
Frolich char	*Salvelinus alpinus erythrinus*	Salmonidae, Salmoniformes
Gilthead sea bream	*Sparus aurata*	Sparidae, Perciformes
Goby	*Pomatoschistus* sp.	Gobiidae, Perciformes
Goby	*Gobiosoma bosci*	Gobiidae, Perciformes
Goldfish	*Carassius auratus*	Cyprinidae, Cypriniformes
Grass carp	*Ctenopharyngodon idella*	Cyprinidae, Cypriniformes
Green chromis	*Chromis caerulea*	Pomacentridae, Perciformes
Grunt	*Haemulon* spp.	Haemulidae, Perciformes
Guppy	*Poecilia reticulata*	Poeciliidae, Cyprinidontiformes
Humbug dascyllus	*Dascyllus aruanus*	Pomacentridae, Perciformes
Jack mackerel	*Trachurus japonicus*	Carangidae, Perciformes
Japanese eel	*Anquilla japonica*	Anguillidae, Anguilliformes
Japanese flounder	*Paralichthys olivaceus*	Paralichthydae, Pleuronectiformes
Japanese pilchard	*Sardinops sagax melanosticta*	Clupeidae, Clupeiformes
Lake char	*Salvelinus namaycush*	Salmonidae, Salmoniformes
Largemouth bass	*Micropterus salmoides*	Centrarchidae, Perciformes
Marbled rockfish	*Sebasticus marmoratus*	Scorpaenidae, Scorpaeniformes
Navaga	*Eleginus navaga*	Gadidae, Gadiformes
Nile tilapia	*Oreochromis niloticus*	Cichlidae, Perciformes
Oriental weatherfish	*Misgurnus anguillicaudatus*	Cobitidae, Cypriniformes
Parrotfish	*Scarus* spp.	Scaridae, Perciformes

Table 1 continued

Common name	Latin name	Family, order
Parrotfish	*Sparisoma* spp.	Scaridae, Perciformes
Pigfish	*Orthopristis chrysopterus*	Haemulidae, Perciformes
Pike	*Esox lucius*	Esocidae, Esociformes
Pinfish	*Lagodon rhomboides*	Sparidae, Perciformes
Plaice	*Pleuronectes platessa*	Pleuronectidae, Pleuronectiformes
Platy	*Xiphophorus maculatus*	Poeciliidae, Cyprinidontiformes
Porgy	*Calamus* spp.	Sparidae, Perciformes
Puffer	*Fugu pardalis*	Tetraodontidae, Tetraodontiformes
Rainbow trout	*Oncorhynchus mykiss*	Salmonidae, Salmoniformes
Reticulated dascyllus	*Dascyllus reticulates*	Pomacentridae, Perciformes
Roach	*Rutilus rutilus*	Cyprinidae, Cypriniformes
Russian sturgeon	*Acipenser gueldenstaedtii*	Acipenseridae, Acipenseriformes
Sea bream	*Chrysophrys (Pagrus) major*	Sparidae, Perciformes
Sea catfish	*Arius felis*	Ariidae, Siluriformes
Sergent-major	*Abudefduf saxatilis*	Pomacentridae, Perciformes
Sharpnose puffer	*Canthigaster valentini*	Tetraodontidae, Tetraodontiformes
Shore rocklings	*Gaidropsarus mediterraneus*	Phycidae, Gadiformes
Siberian sturgeon	*Acipenser baerii*	Acipenseridae, Acipenseriformes
Small puffer	*Sphoeroides sprengleri*	Tetraodontidae, Tetraodontiformes
Smooth dogfish	*Mustelus canis*	Triakidae, Carchariniformes
Snapper	*Ocyurus chrysurus*	Lutjanidae, Perciformes
Stellate sturgeon	*Acipenser stellatus*	Acipenseridae, Acipenseriformes
Striped bass	*Morone saxatilis*	*Moronidae, Perciformes*
Sunfish	*Lepomis macrochirus*	Centrarchidae, Perciformes
Tench	*Tinca tinca*	Cyprinidae, Cypriniformes
Tilapia	*Tilapia zillii*	Cichlidae, Perciformes
Tilefish	*Malacanthus plumieri*	Malacanthidae, Perciformes
Turbot	*Scophthalmus maximus*	Scophthalmidae, Pleuronectiformes
Vendace	*Coregonus albula*	Salmonidae, Salmoniformes
Winter flounder	*Pseudopleuronectes platessa americanus*	Pleuronectidae, Pleuronectiformes
Wrasse	*Thalassoma lunare*	Labridae, Perciformes
Wrasse	*Thalassoma* spp.	Labridae, Perciformes
Wrasse	*Halichoeres* spp.	Labridae, Perciformes
Yellowtail	*Seriola quinqueradiata*	Carangidae, Perciformes
Zander	*Stizostedion lucioperca*	Percidae, Perciformes

surround and separate off the gustatory receptor cells. Up to five basal cells are situated at the basis of the taste bud and have desmosomal attachments to both the gustatory and the supporting cells. External taste buds in some of the Gadidae species have no basal cells (Jakubowski and Whitear 1990).

The nomenclature and interpretation of morphology and function of some cell types are not always consistent. Welsch and Storch (1969) distinguished the elongated cells of the taste buds as 'light' and 'dark' cells. The cells with a single apical microvillus usually appeared less electron-dense than the supporting cells surrounding them; however, in shore rocklings and five-bearded rockling, the supporting cells in the external taste buds are consistently paler than the gustatory receptor cells. The variation of overall electron density of gustatory cells appear to relate to the age of the individual cells (Jakubowski and Whitear 1990). It has been suggested that both the light and the dark cells have synaptic associations with the basal cells as well as with the nerves, and that they are receptor cells (Reutter 1986).

The solitary chemosensory cells have a close cytological resemblance to the gustatory oral and extraoral receptor cells (Whitear 1971; Whitear and Kotrschal 1988; Whitear 1992), and they are also innervated by the facial nerve, as are many taste buds (Whitear and Kotrschal 1988; Kotrschal *et al.* 1993; Kotrschal *et al.* 1998). Both the taste buds and the solitary chemosensory cells have similar primary representation in the facial lobe and also have indistinguishable ascending projections (Kotrschal and Finger 1996). Based on these findings, it was suggested that the solitary chemosensory cells should

© 2003 Blackwell Publishing Ltd,

Table 2 A list of the prey species mentioned in the review

Common name	Latin name	Family, order, and class
Annelide	*Perinereis brevicirrus*	Nereidae, Phylodocida, Polychaeta
Blue crab	*Callinectes sapidus*	Portunidae, Decapoda, Crustacea
Blue starfish	*Coscinasterias tenuispina*	Asteriidae, Forcipulata, Asteroidea
Brine shrimp	*Artemia*	Artemiidae, Anostraca, Crustacea
Copepods	*Eucalanus crassus*	Eucalanidae, Copepoda, Crustacea
Dinoflagellate	*Protogonyaulax tamarensis*	Dinophyta, Dinoflagellata, Flagellata
Duckweed	*Lemna minor*	Lemnaceae, Angiospermae, Monocothyledones
Earthworm	*Eisenia foetida*	Lumbricidae, Opisthopora, Oligochaeta
Krill	*Euphausia pacifica*	Euphausiidae, Euphausiacea, Crustsacea
Mosquito	*Chironomus* sp.	Chironomidae, Diptera, Insecta
Nemertide	*Lineus ruber*	Lineidae, Geteronemertini, Nemertini
Opisthobranch	*Aplysia brasiliana*	Aplysiacea, Tectibranchia, Gastropoda
Polychaete	*Phyllodoce maculata*	Phyllodocidae, Phyllodocida, Polychaeta
Romaine lettuce	*Lactuca sativa*	Asteraceae, Angiospermae, Dicothyledones
Shrimp	*Pandalus borealis*	Pandalidae, Decapoda, Crustacea
Sponge	*Carteriospongia* sp.	Dysidae, Cornacuspongida, Demospongia
Sponge	*Dysidea* sp.	Dysidae, Cornacuspongida, Demospongia
Sponge	*Amphimedon compressa*	Niphatidae, Haplosclerida, Demospongia
Sponge	*Axinella corrugata*	Axinellidae, Cornacuspongida, Demospongia
Sponge	*Ircinia strobilina*	Thorectidae, Dictyoceratida, Demospongia
Sponge	*Hyritios erecta*	Thorectidae, Dictyoceratida, Demospongia
Squid	*Loligo forbesi*	Loliginidae, Decapoda, Cephalopoda

be considered as the gustatory subsystem (Kotrschal 1996, 2000). Regretably, there are not sufficient data about the function of the solitary chemosensory cells to support this hypothesis. Moreover, the density of extraoral taste buds varies with the region at the body surface. In contrast, the solitary chemosensory cells cover the body surface uniformly, and there is no relationship between fish feeding ecology and the mean density of the solitary chemosensory cells. These observations support the view that the solitary chemosensory cells have functions and biological roles distinct from the function of fish taste buds.

Adult taste buds are not static structures. The cells in a taste bud undergo renewal from the epithelial cells that surround the base of the taste bud. These cells undergo division with some of their daughter cells migrating into the taste bud. The average lifespan of a taste cell is dependent on the ambient temperature: in channel catfish, the taste cell turns over every 40 days at 14 °C and every 12 days at 30 °C (Raderman-Little 1979).

The receptor cells and the mucous substances covering the receptor field of a taste bud contain complex polymeric glycoconjugates secreted by the supporting cells (Jakubowski and Whitear 1990), and possibly by the receptor cells (Witt and Reutter 1990) or by the marginal cells (Reutter and Witt 1993). Glycoconjugates are glycoproteins, which are protein chains to which one or more oligosaccharide units are attached. The receptor cells protect the taste buds receptor field mechanically, and probably, also fulfil functions involving taste perception processes. There is little information about the possible function of the mucous substances surrounding the taste buds (Bannister 1974). It has been assumed that the mucous presents a filter system selecting particular substances prior to the chemoreception processes, which take place on the cell membrane of the microvilli on the sensory cells.

Every taste bud has a network of nerves located between the basal cells and the basal part of the receptor cells, which is comprised of unmyelinated and myelinated nerve fibres (Finger *et al*. 1991). The network of nerves is the location of synaptic contacts within the taste bud. Synapses occur at the subnuclear part and at the basal processes of the gustatory receptor cells, and also at basal cells. Nerve fibres innervating taste buds are derived from the facial (VII), glossopharyngeal (IX) and vagal (X) cranial nerves. The facial nerve supplies external taste buds of the barbels and lips (*N. facialis maxillaris*), fins and body surface (*N. facialis recurrens*) and oral taste buds of rostral palate (*N. facialis palatinus*). The vagal nerve

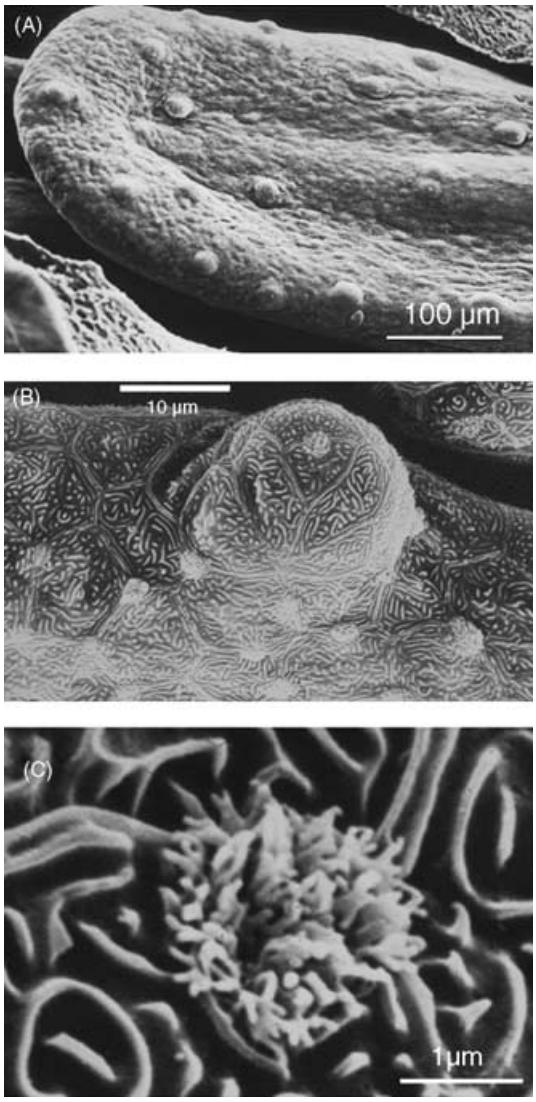

Figure 1 Taste buds on the tongue. (A) Tongue tip of European eel larvae, *Anguilla anguilla* (Anguillidae), showing several papillae. (B) Part of the tongue with one papillae. Several taste pores are seen emerging between epithelial cells with microridges. (C) A taste pore between three epithelial cells. (Scanning electron microscopic pictures by K.B. Døving).

innervates most of the orobranchial taste buds, whereas the glossopharyngeal nerve plays a minor role in the gustatory supply to the oral cavity (Herrick 1901; Atema 1971; Finger 1976). A single nerve fibre may innervate several taste buds, and each receptor cell forms more than one synapse. This suggests a high degree of convergence of input onto the sensory nerve fibres (Kinnamon 1987), which also seems to increase with fish growth (Finger *et al.* 1991).

The primary gustatory nuclei lie in the medulla oblongata, and fish with numerous external taste buds, such as channel catfish, have well-developed facial lobes in the rostral part of the medulla. Cyprinid fish with numerous taste buds in the mouth cavity also possess enlarged primary gustatory nuclei in the vagal lobe regions of the caudal medulla. The glossopharyngeal nerve terminates in a medullar region between the facial and the vagal lobes. Interestingly, in the majority of the fish species, neither the facial nor the vagal lobe is well developed, and the two may even be continuous and grossly indistinguishable from each other. Based on the observed anatomical peculiarities, the gustatory system may be divided into two distinct, though interrelated, subsystems: oral gustatory subsystem and extraoral gustatory subsystem (Finger and Morita 1985).

The gustatory tracts from the facial and vagal lobes ascend and descend within the brain. The major secondary ascending projections from the facial and the vagal lobes in the teleosts studied terminate in a large isthmic nucleus known as the superior secondary gustatory nucleus. Within the medulla, local gustato-motor networks and descending pathways form the neural substrate for reflexive movements such as food pick up or biting and swallowing or rejection (Kanwal and Finger 1992). Presumably, the vagal lobe in cyprinids is involved in stimulus localisation and contains a representation of both gustatory and tactile inputs from the palatal organ in the oral cavity (Morita and Finger 1985). The palatal organ covered by the oral taste buds accomplishes selective ingestion of food by the contraction of specific sets of muscles, which can selectively hold on to 'tasty' food particles (Sibbing *et al.* 1986; Osse *et al.* 1997). A high percentage of bimodal taste/touch neurones are found within the primary gustatory nucleus (Marui *et al.* 1988; Hayama and Caprio 1989). These findings indicate that oral evaluation is based on both chemical and mechanical sensory input.

The functional properties of the fish gustatory system have mainly been studied by electrophysiological means. Hoagland probably obtained the first electrophysiological recording of taste activity in a teleost (Hoagland 1933). Since his pioneering work, a number of reports concerning the physiology of the gustatory system of teleost have appeared (for review, see Marui and Caprio (1992)). The first studies found that the fish gustatory system is highly responsive to many organic and inorganic chemicals as well as to natural food extracts. The responses from the

 © 2003 Blackwell Publishing Ltd,

gustatory nerves are fast adapting or phasic; the peak response is reached within the first few seconds of stimulation and returns to the baseline activity even with continued stimulation. The taste specificities across the fish species studied were very different: different species of fish may have very different rank orders of effective taste stimuli. The estimated thresholds for the most potent substances were less than 10^{-9} M. The dose–response relationships are variously expressed and are different for different taste substances; responses increase sharply with a logarithmic increase in concentration and eventually reach saturation (Marui *et al.* 1983a). The specificities and sensitivities of the taste buds innervated by the different branches of the facial nerve are similar in the same fish species (Caprio 1975; Davenport and Caprio 1982). The oropharyngeal taste buds, which are innervated by both the glossopharyngeal and the vagal nerves, are generally less sensitive to specific amino acids by 1.5–2 orders of magnitude than the taste buds innervated by the facial nerve (Kanwal and Caprio 1983), although they have been shown to have similar amino-acid specificity.

Using the free amino acids and their derivatives as taste substances, it has been found that there are rigid requirements of taste receptors for the molecular structure of the stimulus molecules. For example, gustatory responses to amino acids are highly stereospecific: the L-isomers of amino acids have, for most species studied, been shown to be more stimulatory than its D-enantiomers. Unsubstituted, L-α-amino acids are usually the most efficient compounds for the gustatory system of fish. Neutral amino acids containing two or fewer carbon atoms, and having unbranched and uncharged side chains, are highly stimulatory. Acidic amino acids are poor gustatory stimuli, and basic amino acids are quite variable, depending upon the fish species. The peptides tested so far are less effective than their amino acid residue. Furthermore, the order of the amino acid residues in a simple peptide does not significantly affect its ability to stimulate the gustatory system (Caprio 1978; Hidaka and Ishida 1985; Marui and Kiyohara 1987). It should be noted that there are many exceptions to these findings (Marui and Caprio 1992). A synergistic effect was observed for mixtures of some amino acids in the gustatory system of several fish species (Hidaka *et al.* 1976; Yoshii *et al.* 1979) and, in addition, electrophysiological responses to some amino acids are enhanced by the presence of another amino acid in cross-adaptation experiments (Marui and Kiyohara 1987).

Feeding behaviours mediated by the gustatory system

Various sensory organs can mediate the different feeding behaviours found in fish, and a particular behaviour pattern can be evoked via several sensory systems. For example, the snapping movement with the jaws can be released by stimulation of the lateral line organ or electroreceptors, by visual, auditory or olfactory stimuli or by taste or common chemical sensory stimuli. Other patterns might be more specific, and in some species, a given feeding pattern can be mediated via only one sensory pathway.

Both an extraoral and an oral gustatory subsystem mediate behavioural responses to food items brought in contact with the fish. The behavioural responses are complex and comprise several elements with characteristic features. These response patterns are described and defined below.

Usually, the gustatory systems participate in the final phases of the series of feeding behaviours connected with food search, which normally ends with consumption. The bullhead, for example, may detect, search and successfully find a food source based on information from the external taste receptors (Bardach *et al.* 1967). It seems established that stimulation of the external taste system may mediate grasping, biting, snapping or scraping behaviours, especially in bottom feeders, nocturnal or cave fish, or fish living in deep or turbid water. The presence of numerous external taste buds is common in these fish species (Saxena 1959; Branson 1966; Kapoor *et al.* 1975; Gomahr *et al.* 1992). Unfortunately, experimental evidence supporting the role of extraoral taste reception in these types of feeding behaviours is scarce. Most studies have been concerned with the evaluation of the gustatory system for processing of food during the oral phase.

Extraoral taste response: when a fish with a well-developed extraoral taste system touches a food object, the fish makes an abrupt effort to reach the object or to avoid it. The fish can stop, turn around, turn to the side, swim backwards, initiate searching trajectories or make circles and S-shaped loops to reach the object. Finally, snapping with the jaws at high frequency might follow a touch of a food item. In sturgeon, the series consists of snapping with the jaws two or more times. This species has no object vision, but searches the bottom surface thoroughly and collects all appropriate food items using the snapping behaviour. There is a dramatic increase in the bottom-surface snapping in various fish species

upon stimulation by food odour (Atema *et al.* 1980; Kasumyan and Ponomarev 1986; Kasumyan 1999a). This pattern of feeding behaviour is also evident in fish with a well-developed visual system; for example, rainbow trout and brown trout (Døving and Kasumyan, unpublished).

Once the food item is in a proper position in relation to the mouth of the fish, the fish grasps or snaps the object with the jaws. Food objects containing aversive substances cause fish to neglect the object by moving forward, as do sturgeons, or change their searching direction, as do channel catfish. While incitants increase the snap frequency, suppressants decrease the frequency of snapping movements.

Oral taste response: food objects can be taken into the mouth by scraping, tearing, sucking, snapping and grasping (Nikolski 1963; Wootton 1998). The behaviour pattern most intensively studied in conjunction with feeding is the sucking and snapping movement in fish (for review, see Osse *et al.* 1997). This behaviour consists of a series of movements that involves a number of anatomical structures and a sequence of finely adjusted muscle activity (Fryer and Iles 1972; Liem 1973).

The snapping movements performed with the jaws are extremely fast in anglerfish, which attract preys with movable illicium (Grobecker and Pietsch 1979). Furthemore, in species of other families, such as gadids and salmonids, these movements are so fast that they are difficult to observe in detail without the use of suitable recording equipment.

Motta (1982, 1988) and Coughlin and Strickler (1990) have studied particulate feeding behaviour on plankton. Recordings of the prey capture mechanisms used by the blue-green chromis, while feeding on calanoid copepods and brine shrimp reveals that it is capable of a ram-jaw, low-suction feeding, as well as a typical suction feeding behaviour. By adjusting the degree of jaw protrusion and the amount of suction used during a feeding strike, they can modulate feeding strikes according to the prey type being encountered. The ram-jaw feeding mode enables blue-green chromis to capture highly evasive calanoid copepods within 6–10 ms (Table 2).

When a food object is in the mouth, it is subject to final sensory judgements. As a result of these oral tests, the food item can then be rejected or swallowed. The time for which the object is kept in the mouth before swallowing or rejection is called the retention time. During the retention time, the fish detect and recognise taste substances, assess the palatability of the food object and perform the decision to swallow or to spit it out. For many fish, even if an object is newly spat out, it can be recaught. This sequence can be repeated several times. The retention time may be determined for each recatch, and sum of the retention times may be calculated. The duration of a taste response is usually relatively short and ends by swallowing a food object or by ignoring it when fish lose interest in a food object and do not recapture it.

During the retention time, a putative food can be sorted into waste material and acceptable items. The food is masticated and thereafter transported via the orobuccal cavity. This period can be long and is not necessarily followed by acceptance and swallowing. As studied in detail in the common carp and other cyprinids, the food selection procedure comprises repetitive positioning of the food and transport movements. Food is transported through the posterior side of the palatal organ and the postlingual organ of fish, where the density of oral taste buds reaches a maximum value of 820 mm^{-2} in bream (Osse *et al.* 1997). During the process of taste selection, food items, most probably, are fixed between the palatal organ as roof and the postlingual organ as bottom, and rinsed by the alternating flow of water, while waste materials are expelled through the branchial slits (Sibbing *et al.* 1986). In the complex mastication process, the food is crushed and ground to small and acceptable pieces (Sibbing 1988; Sibbing 1991). A mollusc feeder, Alluaudi cichlid, uses powerful teeth to destroy the shell of the mollusc and then spits out up to 90% of the fragments of the shell without loss of flesh (Hoogerhoud 1989).

Benthivorous and herbivorous fish keep the food in the mouth for much longer time than piscivorous fish. Even though predators have a short retention time, the gustatory system still controls the feeding responses. The taste judgements made during the oral examination is an obligatory phase of feeding behaviour in every fish species, irrespective of the feeding strategies used or the extent to which the gustatory system is developed.

Classification of taste substances

Chemical substances can be divided into several categories or types, according to their effects on the feeding behaviour of fish. The nomenclatures used for classifications of chemical stimuli which evoke feeding behaviour are varied (Lindstedt 1971; Mackie 1982; Mearns *et al.* 1987; Sakata 1989). We will emphasise the distinction between the substances that have

 © 2003 Blackwell Publishing Ltd,

effects on the oral and extraoral taste systems. These systems play different roles in fish feeding behaviour (Atema 1980; Pavlov and Kasumyan 1990) and differ in their functional characteristics (Marui and Caprio 1992; Kasumyan 1999a). The presence or absence of these compounds in the diet determines whether a food item is grasped or ignored, is eaten or rejected and, to some extent, how much of the food is consumed.

'Incitants' are substances that induce capture of the food item via the extraoral taste system. There are a number of different behaviours that can presumably be evoked by incitants like: suction, grasping, snapping, biting, tearing or pinching.

'Suppressants' are substances that decrease the rate of grasping food items. Like incitants, suppressants are mediated by the extraoral gustatory system and control the rate of grasping food items.

'Stimulants' are substances characterised by high ingestion rate. This behaviour is evoked by the oral taste system. Stimulants promote fish feeding. Usually, fish swallow the food items containing stimulants at first capture. This behaviour is mediated by the oral gustatory system.

'Deterrents' are substances that make the fish abandon food intake and evoke food rejection. Deterrents are characterised by high rejection and low ingestion of food items that are caught. Often, fish feeding motivation is decreased for a short period after a fish has tested a food item that contains a deterrent. Usually, the retention time of food items that contain a deterrent substance is short and the fish does not attempt to recatch the food item. This behaviour is mediated by the oral gustatory system.

'Enhancers' or potentiators are substances that, although not feeding stimulants *per se,* accentuate the flavour of the food and cause fish to increase their consumption of flavoured food. In some cases, however, stimulants may be ineffective as feeding enhancers. This behaviour is evoked by the oral taste system.

Presumably, there should also be another category of taste substances, 'detractors', which essentially are neutral substances that diminish the positive effects of the stimulants. Unfortunately, appropriate examples of substances that belong to this category of taste stimuli do not yet exist.

As will be shown, a single substance might have different effects on the taste behaviour of the same fish and can be both incitant and deterrent, or incitant and stimulant. A number of substances do not induce any effects on fish behaviour either via the extraoral or the oral taste systems and are classed as 'indifferent' substances.

Methods in studies of fish taste preferences

Several methods for presenting and quantifying fish taste preferences have been developed as bioassays. Space does not permit us to discuss these methods at length. Many of these methods, though interesting and valuable, have limitations because they do not make it possible to distinguish between the taste and the olfactory systems. Examples are the methods developed by Carr and coworkers (Carr 1976; Carr and Chaney 1976; Carr *et al.* 1977). Likewise, Sutterlin and Sutterlin (1970) used polyurethane discs impregnated with various substances for studying taste preferences in Atlantic salmon. Jones (1989, 1990) used cotton pellets soaked in solutions of test substances in attempts to study taste preferences in rainbow trout.

Several authors have used a solid matrix carrying the stimulants. A few authors have used purified starch gel as a carrier for test substances (Hidaka *et al.* 1978; Ohsugi *et al.* 1978; Murofushi and Ina 1981; Hidaka 1982; Kasumyan 1992; Kasumyan and Kazhlaev 1993a). Mearns *et al.* (1987) developed another approach using agar gel as carrier for taste substances. This method was elaborated by Kasumyan and collaborators (Kasumyan and Sidorov 1993a,b, 1995c,d; Kasumyan and Morsy 1996, 1997). Their results showed that this method distinguished between smell and taste (Table 3), and they proposed the index of palatability as a quantitative estimation of taste preference (in percent):

$$I_{pal} = 100 \cdot (R - C) \cdot (R + C)^{-1}$$

where R is the consumption of flavoured pellets and C is the consumption of unflavoured pellets. The I_{pal} is an analogy with the index of electivity used by Ivlev (1961).

Large disk-shaped agar gel (1.5 cm in diameter and about 2.0 cm in length) were used to present test compounds for studying taste preferences in herbivorous tilapia, but experiments on anosmic fish were not made using this test procedure (Johnsen and Adams 1986; Adams *et al.* 1988). Other bioassay procedures include incorporating various compounds into freeze-dried pellets (Mackie and Adron 1978; Mackie 1982). Radiographic (Talbot and Higgins 1983; Talbot 1985; Toften *et al.* 1995) or radioisotopic methods (Storebakken *et al.* 1981; Jobling *et al.* 1995) have also

Table 3 Comparing taste responses in intact and anosmic fish

	Concentration (mM)	Acceptance ratio (%)	Palatability index (%)	Number of snaps	Retention time (s)		Number of trials
					First capture	All captures	
Intact fish	100	100***	59	1.0***	15.3***	15.3***	48
	10	49.9*	31.8	1.4	9.3**	12.4	48
	1	28.3	4.6	1.5	7.6	9.8	48
	0	25.8	–	1.8	4.4	7.8	48
Anosmic fish	100	100***	48.1	1.0	14.4***	14.4***	20
	10	60*	26.3	1.8	7.9	11.7	20
	1	35	0	1.4	8.2	9.0	20
	0	35	–	1.9	4.3	9	20

Properties of taste responses to different concentrations of L-cysteine in intact and anosmic carp *Cyprinus carpio* (9–12 cm body length). The experiments with anosmic fish were performed 10 days after operation. See text for details. Data modified from Kasumyan and Morsy (1996).

In this and the following tables, the significance levels are indicated as follows: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$. The control pellets were made of 2% agar and contained the red colour Ponceau 4R at a concentration of 50 μM. The Chi-square test was used for estimation of acceptance ratio and Student's *t*-test for all other characteristics of taste responses.

been used for comparison of the palatability of food materials for fish. A special approach was developed for the assessment of taste preferences and a distinction between oral and extraoral taste systems (Kasumyan 1992; Kasumyan *et al.* 1992; Kasumyan and Kazhlaev 1993a).

Taste preferences for different types of substances

It has been shown that chemical substances evoke different types of behavioural taste responses, thus they are perceived as different by fish. The assessment of palatability has been investigated for free amino acids and their derivatives, classical taste substances, nucleotides and nucleosides, quaternary amines, organic acids and other types of substances. In the following sections, we summarise data available concerning fish taste preferences to these substances and their mixtures.

Classical taste substances

Man almost exclusively confined earlier investigations on gustation in fish to classical taste substances, representing what has been considered the basic taste sensations recognised. They are considered to be sweet, sour, bitter and salty, represented, for example, by sucrose, acetic acid, quinine and sodium chloride. von Uexküll (1895) made the first study of fish taste preference to classical taste substances on smooth dogfish. There have been a limited number of studies performed on taste preferences in fish to classical taste substances, and they are difficult to compare, as they were obtained using various behavioural assays and different carriers for the chemicals.

Sucrose: it has been found that the European minnow can be trained to distinguish among sugar solutions and solutions of sour, bitter and salty substances (Strieck 1924). Trudel (1929) was able to train European minnows to distinguish between several natural sugars, and Krinner (1934) was able to train European minnows to distinguish between sodium chloride and saccharose. Conditioned European minnows are able to discriminate between saccharose and saccharin at low concentrations (Glaser 1966). The chemosensory system mediated fish responses to a solution of the substance, as conditioned stimuli were not defined in these studies.

Studies, where appropriate methods were used, show that sucrose is usually an indifferent taste substance for fish or evokes a positive oral taste response. Sucrose is palatable for many herbivorous fish like grass carp and is palatable for omnivorous fish in which algae is the main component of the diet. Examples are dace, roach, guppy, black molly and platy (Andriashev 1944; Appelbaum 1980; Kasumyan 1997; Kasumyan and Morsy 1997; Kasumyan and Nikolaeva 1997, 2002; Tables 4 and 5). Of 36 fish species tested by different authors, sucrose was found to be a stimulant for 15 species and was an indifferent

 © 2003 Blackwell Publishing Ltd,

taste substance for 18 mostly carnivorous species. Sucrose evokes a deterrent taste response in one fish species only, namely the puffer (Hidaka 1982). According to Appelbaum (1980), common carp also released a negative taste response to food containing sucrose; however, as was shown using another behavioural approach, sucrose is an indifferent taste substance for common carp, at least at a concentration of 0.29 M (10%; Kasumyan and Morsy 1996).

Acids: it was shown that food with 0.1 M HCl is rejected by the puffer (Hidaka *et al.* 1978) and the shore rockling (Andriashev 1944). The pike, the zander and the vendace rejected food containing citric acid (Appelbaum 1980). Atlantic salmon consumed little food that has a sour taste (Stradmeyer 1989). In contrast, acid food was willingly consumed by tilapia (Adams *et al.* 1988), Nile tilapia, European eel and rainbow trout (Appelbaum 1980). In a number of fish species, citric acid is a highly efficient stimulant, in others it is a strong deterrent (Kasumyan 1997; Tables 4 and 5). In total, 37 fish species were investigated concerning their taste preferences to diet containing citric acid or other acids. For 16 fish species, acids strongly depressed food acceptance, and for the other 15 fish species, the palatability of food was highly increased after the addition of acid to the diet. Citric acid was an indifferent taste substance for eight fish species only. It is noteworthy that acidified diets are palatable mostly for salmonids and poecilids, but evoke a negative taste response in acipenserids and in many cyprinids.

Quinine, calcium chloride and related compounds: quinine is a highly deterrent substance for all fish species tested. Smooth dogfish rejected mackerel impregnated with quinine immediately after the food had been snapped up (von Uexküll 1895). Sheldon (1909) found that a solution of quinine hydrochloride produced jerk responses and other movements in the smooth dogfish, when applied to the inside of the mouth and spiracle, but not when applied to other parts of the body. Evidently, this bitter-tasting substance elicited responses only from the regions that bear the taste buds. Quinine solution applied extraorally had released an avoidance response in some teleost fish (Scharrer *et al.* 1947), and Hidaka *et al.* (1978) reported that quinine inhibited food consumption in the puffer. Quinine and several other bitter-tasting compounds like papaverine, theophylline and caffeine inhibited the feeding behaviour of the turbot (Mackie 1982). Quinine, papaverine, sodium ricinoleate and sodium taurocholate, all of which taste bitter to humans, were found to be feeding deterrents for the Dover sole (Mackie and Mitchell 1982b). In goldfish, gelatine pellets flavoured with quinine or caffeine were strongly rejected (Lamb and Finger 1995). The shore rockling rejected meat soaked in warmot extract (*Artemisia* sp. (Asteraceae); Andriashev 1944) and wild goldfish rejected pellets containing calcium chloride (Kasumyan and Nikolaeva 2002).

For humans, calcium chloride has a bitter and astringent taste. In contrast to quinine, calcium chloride has been found to be an indifferent taste substance for 17 fish species, a deterrent for three species and a stimulant for seven species (Tables 4 and 5).

Sodium chloride: sodium chloride is either an indifferent taste substance or a stimulant for fish (Tables 4 and 5). The addition of sodium chloride to starch gel or to starch gel containing synthetic clam extract did not enhance or reduce the pellet consumption by fugu (Hidaka 1982). Shore rockling rejected meat pieces soaked in 10% sodium chloride (Andriashev 1944). A high level (60%) of sodium chloride made artificial food unpalatable for European eel, but the taste response of rainbow trout was positive for sodium chloride at a very high concentration (80%; Appelbaum 1980).

Free amino acids

The food of fish consists of a variety of prey organisms and aquatic plants. All these feeds contain considerable amounts of free amino acids (Dabrowski and Rusiecki 1983; De la Noue and Choubert 1985; Holm and Walter 1988; for review, see Carr *et al.* 1996). Fish taste receptors are highly sensitive to free amino acids and induce strong electrophysiological responses in recordings of the nervous activity from gustatory fibres (for review, see Marui and Caprio 1992). Thus, free amino acids have been found to be highly efficient incitants or stimulants for various freshwater and marine fish (Hidaka 1982; Mackie 1982; Mackie and Mitchell 1983; Mearns *et al.* 1987; Adams *et al.* 1988; Jones 1989; Lamb and Finger 1995). In some species, the mixtures of free amino acids alone had the same level of palatability as the whole extract of preferable food organisms (Fuke *et al.* 1981; Mackie 1982; Takeda *et al.* 1984; Johnsen and Adams 1986; Mearns *et al.* 1987).

For European eel, a mixture of neutral and acidic amino acids as well as neutral amino acids only were the most palatable, while aromatic and basic amino acids were inactive (Mackie and Mitchell 1983). The same conclusions were made after testing various

Table 4 The index of palatability to classical taste substances for 27 different fish species

Substances	Concentration, mM (%)	Brown trout, *Salmo trutta caspius*	Brook char, *Salvelinus fontinalis*	Lake char, *Salvelinus namaycush*	Frolich char, *Salvelinus alpinus erythrinus*	Chum salmon, *Oncorhynchus keta*	Common carp, *Cyprinus carpio*	Crucian carp, *Carassius carassius*	Goldfish, *Carassius auratus*	Roach, *Rutilus rutilus*
Citric acid	0.26 (5)	78.7***	95.2***	60.0***	53.4***	−100***	53.9***	−61.2***	−31.0***	−100**
Sodium chloride	1.73 (10)	43.5***	60.7	48.4***	12.6	12.3	22.4	0.5	−15.9*	32.7*
Calcium chloride	0.9 (10)	37.7**	87.7***	46.7***	17.7	−7.8	30.4**	6.1	−14.4*	21.8
Sucrose	0.29 (10)	−10.2	90.6***	11.2	9.7	−14.7	−13.8	0	1.9	32.7*
		Dace, *Leuciscus leuciscus*	Chub, *Leuciscus cephalus*	European minnow, *Phoxinus phoxinus*	Tench, *Tinca tinca*	Bitterling, *Rhodeus sericeus amarus*	Grass carp, *Ctenopharyngodon idella*	Tiger barbus, *Puntius tetrazona*	Zebrafish, *Brachydanio rerio*	Nine-spined stickleback, *Pungitius pungitius*
Citric acid	0.26 (5)	17.1	−9.8	10.3	56.4***	−89.1***	46.0***	−18.5	100	66.7***
Sodium chloride	1.73 (10)	24.4*	17.9	7.9	36.8***	−6.3	−5.1	11.0	100	−10.4
Calcium chloride	0.9 (10)	9.2	44.0***	14.8	33.4***	−7.4	−20.9	15.7	0	−11.0
Sucrose	0.29 (10)	28.6**	−1.3	7.5	10.6	5.6	63.4***	10.0	100*	−0.7
		Atlantic wolffish, *Anarhichas lupus*	Banded cichlid, *Heros* severum	Black molly, *Poecilia sphenops*	Guppy, *Poecilia reticulata*	Platy, *Xiphophorus maculatus*	Siberian sturgeon, *Acipenser baerii*	Stellate sturgeon, *Acipenser stellatus*	Arctic flounder, *Liopsetta glacialis*	Navaga, *Eleginus navaga*
Citric acid	0.26 (5)	42.3*	−22.0**	1.3	95.2***	77.2***	−89.5**	−89.5**	−11.3*	28.9
Sodium chloride	1.73 (10)	35.3	−73.5***	33.4***	−100	−87.1***	−50.0**	−47.5**	9.2*	−100
Calcium chloride	0.9 (10)	31.1	21.5***	−19.3	72.4	23.6	−100***	−83.7**	6.9	9.5
Sucrose	0.29 (10)	3.3	−13.5	53.5***	83.7***	74.5***	−12.5	−7.1	2.0	20.3

The index of palatability (in percent) was calculated by the formula $I_{pal} = 100 \cdot (R - C) \cdot (R + C)^{-1}$, where R is the number consumed of pellets containing a particular substance and C is the number of blank pellets consumed. See text for details.

© 2003 Blackwell Publishing Ltd.

Table 5 Taste reactions to different substances

Substances	Concentration mM (%)	Stimulants	Deterrents
Alanine	0.1	12	2
Cysteine	0.1	9	5
Serine	0.1	8	2
Glutamine	0.1	7	1
Glycine	0.1	7	1
Threonine	0.1	6	3
Histidine	0.1	6	4
Proline	0.1	6	4
Arginine	0.1	6	6
Valine	0.1	6	6
Phenylalanine	0.1	5	5
Asparagine	0.1	4	2
Methionine	0.1	4	5
Lysine	0.1	3	4
Norvaline	0.1	3	2
Glutamic acid	0.01	7	3
Triptophan	0.01	6	2
Aspartic acid	0.01	6	3
Isoleucine	0.01	3	3
Leucine	0.01	3	3
Tyrosine	0.001	7	2
Citric acid	0.26 (5)	11	9
Sodium chloride	1.73 (10)	7	5
Calcium chloride	0.9 (10)	7	3
Sucrose	0.29 (10)	8	0

The assessment of the palatability of amino acids (L-isomers) and classical taste substances based upon trials on different fish species. The number of fish species examined was 20 for cysteine and norvaline and 21 for the other amino acids, and 27 for classical taste substances. The assessment of palatability of taste substances was made in comparison to blank pellets.

Table 6 Stimulants and deterrents

Species	Stimulants	Deterrents
Platy (*Xiphophorus maculatus*)	13	2
Tench (*Tinca tinca*)	12	0
Chum salmon (*Oncorhynchus keta*)	12	2
Black molly (*Poecilia shenops*)	8	11
Goldfish (*Carassius auratus*)	8	3
Roach (*Rutilus rutilus*)	8	0
Dace (*Leuciscus leuciscus*)	8	0
Siberian sturgeon (*Acipenser baerii*)	7	1
Russian sturgeon (*Acipenser gueldenstaedtii*)	6	0
Common carp (*Cyprinus carpio*)	6	7
Navaga (*Eleginus navaga*)	6	7
Brown trout (*Salmo trutta caspius*)	6	5
Guppy (*Poecilia reticulata*)	5	0
Frolich char (*Salvelinus alpinus erythrinus*)	4	1
European minnow (*Phoxinus phoxinus*)	4	3
Bitterling (*Rhodeus sericeus amarus*)	4	9
Stellate sturgeon (*Acipenser stellatus*)	3	0
Grass carp (*Ctenopharyngodon idella*)	3	17
Chub (*Leuciscus cephalus*)	1	0
Crucian carp (*Carassius carassius*)	0	0
Arctic flounder (*Liopsetta glacialis*)	0	0

The number of amino acids that acts as stimulants or deterrents in 21 fish species. The number of amino acids (L-isomers) tested was 21 for all species except the Siberian sturgeon that was tested for 19 amino acids.

mixtures of different amino acids for European sea bass, where it was also shown that the nonamino acid components of synthetic squid extract were ineffective as taste stimulants (Mackie and Mitchell 1982a).

Not all amino acids, however, are palatable for fish. In a series of experiments performed by Kasumyan and collaborators, the oral taste responses evoked by common free amino acids were investigated for 21 fish species (reviewed in Kasumyan 1997; Table 6). The data reveal that the number of amino acids that acted as stimulants ranged from 0 to 13. There are only a few fish species for which the number of stimulatory amino acids reaches more than 10. For 13 fish species, the number of stimulatory amino acids was six or less. On an average, for all fish species tested, 28% of the free amino acids were stimulatory. The range of free amino acids acting as incitants for fish is wide and includes 14, 15 and 19 amino acids, as was found for three species of the genus *Acipenser* (Kasumyan and Sidorov 1994; Kasumyan 1999a).

The spectra of stimulatory free amino acids are highly species-specific, and the list of palatable amino acids is different for different fish species (for details see section 'Species specificity'; Table 7). The amino acids L-alanine, L-cysteine and L-serine act most frequently as a stimulant for 21 fish species (Table 5). In addition, the L-alanine and L-serine were palatable for sea bream (Goh and Tamura 1980a), tilapia (Johnsen and Adams 1986) and puffer (Hidaka 1982). The amino acids L-glutamine, glycine, L-glutamic acid, L-tyrosine, L-arginine, L-histidine, L-threonine, L-valine, L-tryptophan and L-aspartic acid followed as stimulants in that order after L-alanine, L-cysteine and L-serine; L-lysine, L-norvaline, L-leucine and L-isoleucine were rarely observed as stimulants (Table 5); however, some of these amino acids, L-leucine and L-isoleucine, were stimulants for rainbow trout (Jones 1989).

The essential amino acids, which are important as nutrients for most animals, are also a requirement for many fish species (Millkin 1982). However, the

Table 7 The index of palatability to free amino acids for 21 different fish species

Amino acid (L-isomers)	Concentration (mM)	Brown trout, *Salmo trutta caspius*	Frolich char, *Salvelinus alpinus erythrinus*	Chum salmon, *Oncorhynchus keta*	Common carp, *Cyprinus carpio*	Crucian carp, *Carassius carassius*	Goldfish, *Carassius auratus*	Roach, *Rutilus rutilus*
Alanine	100	−6.6	5.1	38.7***	39.6**	−1.2	17.1	39.4**
Arginine	100	31.6	9.8	−20.9*	−59.8*	0	26.5**	44.5***
Asparagine	100	−68.8	−5.9	−6.3	−33.0	−2.3	−28.2	15.3
Cysteine	100	87.4***	63.2***	−39.3***	74.1***	−11.6	−5.1	−17.6
Glutamine	100	−100*	−5.9	12.7	39.2**	−1.1	−33.0	34.3*
Glycine	100	−100*	0	16.7	−26.7	−1.1	36.1***	28.7
Histidine	100	58.7**	37.9*	22.0***	−14.0	−0.6	−30.8	7.7
Lysine	100	0.8	−79.9**	−5.3	−21.0	−4.6	−28.2	23.1
Methionine	100	−100*	0	30.4***	−84.9**	−3.4	−36.0*	14.5
Norvaline	100	−100*	5.1	15.1	−38.7	−4.6	−28.2	37.0**
Phenylalanine	100	52.9**	14.2	37.5***	−87.3***	−0.6	−42.9*	0
Proline	100	−0.8	9.8	22.6***	55.7***	0.6	−64.1**	5.0
Serine	100	−71.1	0	27.1***	−87.3***	−1.2	26.1**	46.3***
Threonine	100	−54.0	−5.9	7.3	−61.5**	−2.3	35.2***	41.7***
Valine	100	−100*	−12.6	17.1*	−100***	−2.8	31.2***	18.7
Aspartic acid	10	67.3***	65.6***	−2.2	40.7**	−1.1	20.4	−35.8
Glutamic acid	10	29.0	65.2***	14.3*	42.3***	0.6	8.1	7.7
Isoleucine	10	−100*	−5.9	35.3***	−36.1	0.6	33.5***	−19.0
Leucine	10	−39.8	27.8	39.5***	−23.0	0.6	39.2***	21.7
Tryptophan	10	67.7***	18.1	14.9*	−51.5*	0	−13.3	35.5*
Tyrosine	1	76.0***	0	29.3***	−24.6	−1.1	37.4***	32.1*

		Dace, *Leuciscus leuciscus*	Chub, *Leuciscus cephalus*	European, minnow *Phoxinus phoxinus*	Tench, *Tinca tinca*	Bitterling, *Rhodeus sericeus amarus*	Grass carp, *Ctenopharyngodon idella*	Black molly, *Poecilia sphenops*
Alanine	100	64.3*	52.0**	26.3*	79.2***	11.9***	−49.6*	31.5***
Arginine	100	39.4	0	−54.0**	57.1***	4.9	−66.4**	−39.6***
Asparagine	100	31.5	−14.1	−6.4	38.2*	0	−100***	−32.6***
Cysteine	100	57.2*	33.3	−8.8	87.1***	−53.1***	43.4***	−25.9**
Glutamine	100	48.4	21.2	27.9*	49.6**	−18.6***	−7.9	28.6***
Glycine	100	58.6*	0	20.0	43.2*	−5.8	28.4**	−14.7
Histidine	100	−33.3	−23.8	−51.9*	35.6	−58.8***	−87.4***	−70.0***
Lysine	100	−2.4	0	−37.8	56.5***	6.5*	−85.4***	−67.1***
Methionine	100	31.5	36.5	13.8	54.1***	6.1*	−84.5**	28.2***
Norvaline	100	65.5**	29.8	15.4	22.5	−2.3	−86.4***	29.7***
Phenylalanine	100	41.0	−100	−65.5**	2.9	−26.3***	−84.5**	43.7***
Proline	100	57.2*	40.0	31.2*	74.6***	−18.6***	−100***	−29.0**
Serine	100	62.3*	38.0	17.5	68.5***	0	−74.5**	29.7***
Threonine	100	50.0	37.1	−14.2	17.3	−10.1**	−100***	38.9***
Valine	100	73.1***	30.9	25.9*	38.0*	−29.4***	−72.0**	−61.9***
Aspartic acid	10	2.2	−4.0	−18.1	45.1**	−14.2***	36.4**	−32.6***
Glutamic acid	10	59.8*	−4.0	−16.7	26.4	1.0	−66.4**	−24.3**
Isoleucine	10	44.6	−50.1	−14.2	−1.5	−1.8	−100***	−29.3***
Leucine	10	−2.4	−46.6	−2.6	0	−7.5*	−85.4**	−25.9***
Tryptophan	10	39.4	10.4	1.4	−32.7	2.7	−88.4***	−3.0
Tyrosine	1	−35.2	−52.9	−20.5	−3.1	7.3**	−100***	21.6**

© 2003 Blackwell Publishing Ltd,

Table 7 continued

		Guppy, *Poecilia reticulata*	Platy, *Xiphophorus maculatus*	Siberian sturgeon, *Acipenser baerii*	Russian sturgeon, *Acipenser gueldenstaedtii*	Stellate sturgeon, *Acipenser stellatus*	Arctic flounder, *Liopsetta glacialis*	Navaga, *Eleginus navaga*
Alanine	100	−100	59.7***	−16.3*	−19.4	58.5***	3.3	72.1***
Arginine	100	−100	27.5*	12.6*	28.9*	−15.4	2.6	−100*
Asparagine	100	−100	43.9***	12.5*	20.5	14.2	2.6	53.7**
Cysteine	100	77.5*	−100***	–	55.3***	56.1***	−0.9	−100*
Glutamine	100	95.3***	26.1*	−7.9	21.7	−13.8	4.3	35.8
Glycine	100	97.9***	16.9	18.9***	19.4	35.5	2.6	46.8*
Histidine	100	−100	55.7***	14.0*	8.9	57.0***	5.8	−24.7
Lysine	100	77.8*	−30.4	−11.1	25.6	30.5	−3.6	−100*
Methionine	100	−100	−100***	6.7	4.0	−3.3	2.6	37.6
Norvaline	100	0	0.4	–	−20.9	−3.0	2.6	35.8
Phenylalanine	100	−100	36.7**	6.0	27.6	1.0	2.6	74.8***
Proline	100	50.0	46.9***	−3.1	−9.7	−15.0	2.6	−18.3
Serine	100	−100	41.4***	−1.6	29.4	20.1	2.6	50.3*
Threonine	100	−100	42.2***	28.2***	30.6*	4.0	6.6	18.3
Valine	100	0	−1.5	14.6*	19.1	−15.0	6.6	−100*
Aspartic acid	10	0	18.4	5.9	49.6***	17.6	6.6	−100*
Glutamic acid	10	96.1***	−5.1	18.0**	36.9*	−37.1	0.9	−100*
Isoleucine	10	−100	46.8***	3.8	−6.2	1.6	2.6	34.9
Leucine	10	−100	40.4***	11.1	−3.4	−1.9	2.6	34.9
Tryptophan	10	0	37.6***	4.2	39.4**	−1.3	2.6	61.1***
Tyrosine	1	−100	72.5***	−1.0	−4.5	−4.7	0	−100*

palatable amino acids were not always among these essential amino acids. In common carp, none of the essential amino acids were among the palatable substances (Kasumyan and Morsy 1996).

The amino acids are indifferent taste substances for fish more often than they are stimulants. In particular, L-norvaline, L-leucine, L-lisoleucine, L-asparagin, L-lysine, L-tryptophan, L-glutamine and glycine are mostly indifferent taste substances for fish (Tables 5 and 7). L-Alanine, L-asparagine, L-aspartic acid, L-glutamine, glycine, L-histidine, L-serine, L-threonine and L-valine (all in 0.1–1.0 M) did not have stimulatory or deterrent effect for rainbow trout (Jones 1989), whereas tilapia, red sea bream and puffer (Goh and Tamura 1980a; Hidaka 1982; Johnsen and Adams 1986) had no reaction to a range of amino acids. The majority of amino acids did not have any effect on oral taste responses in chum salmon, roach, Siberian sturgeon, Russian sturgeon, guppy, frolich char, European minnow and bitterling. The number of amino acids classed as indifferent taste substances was 16 in guppy and frolich char, 18 in stellate sturgeon and 20 in chub. Moreover, none of the amino acids were found to be stimulatory for crucian carp and Arctic flounder (Table 7). This corresponds well with data obtained for turbot in which it was found that the L-amino acid component of a synthetic squid mixture was totally inactive as taste stimulant for this fish, but the nonamino acid components were highly stimulatory (Mackie 1982). Ikeda *et al.* (1988b) showed that for jack mackerel, only L-tryptophan, among 20 free amino acids tested, had a positive effect on diet consumption. The amino acids fraction of a synthetic krill elicited low feeding activities in marbled rockfish (Takaoka *et al.* 1990) and a mixture of several free amino acids including alanine, glycine, proline, serine, leucine, valine, histidine and tryptophan was ineffective as feed enhancers for juvenile largemouth bass (Kubitza *et al.* 1997).

Amino acids might have an aversive taste for fish and thus function as deterrents. Some of these substances significantly decrease the palatability of diets. Of the amino acids examined by Mackie (1982) in rainbow trout, L-proline and a mixture of L-taurine, L-alanine and L-arginine were deterrents. Among 21 fish species investigated by Kasumyan and collaborators, deterrent amino acids were found for 12 species. Four of these fish species responded negatively for one or two amino acids only; however, for some fish, the number of deterrent amino acids was high, being 7–11 for bitterling, common carp, navaga and black molly and 17 for grass carp

(Table 6). Deterrent taste properties are characteristic for L-valine, L-arginine, L-phenylalanine, L-methionine, L-cysteine, L-lysine, L-histidine and L-proline (Table 5). Many of the deterrent amino acids are highly aversive taste stimuli. Pellets containing these substances were rejected by fish in 100% of the trials. Pellets contained arginine, cysteine, lysine, valine, aspartic acid, glutamic acid and tyrosine, when fed to navaga, and glutamine, glycine, methionine, norvaline, valine and isoleucine, when fed to brown trout. Chubs rejected phenylalanine, while valine was rejected by common carp; asparagine, proline, threonine and isoleucine were rejected by grass carp and cysteine and methionine were rejected by platy (Table 7).

The taste properties of amino acids are highly stereospecific. D-isomers of amino acids are usually deterrent or indifferent types of taste substances, while the corresponding L-forms might be highly palatable for the same fish species, as shown for rainbow trout (Adron and Mackie 1978), plaice (Mackie 1982), European sea bass (Mackie and Mitchell 1982a) and European eel (Mackie and Mitchell 1983). The stereospecificity of amino acids as gustatory stimuli was shown by electrophysiological methods in many studies (Caprio 1978; Yoshii *et al.* 1979; Kiyohara *et al.* 1981; Marui *et al.* 1983a,b; Marui and Kiyohara 1987); however, as was shown for the sea catfish, there is a group of taste fibres in the facial taste system that is more responsive to D-alanine than to its L-enantiomer (Michel and Caprio 1991).

Betaine

Betaine (glycine betaine, trimethylglycine) is widely distributed in fish food organisms (Konosu *et al.* 1966; Konosu and Hayashi 1975; for review, see Carr *et al.* 1996). The gustatory system of many fish species is highly sensitive to this substance (Yoshii *et al.* 1979; Goh and Tamura 1980b; Marui *et al.* 1983a,b; Ishida and Hidaka 1987; Hara *et al.* 1999).

Using bioassays, betaine has been identified as a stimulant for puffer (Hidaka 1982) and Dover sole (Mackie *et al.* 1980; Mackie and Mitchell 1982b). However, many fish do not show positive taste response to betaine. Betaine was an indifferent taste substance for red sea bream (Goh and Tamura 1980a), plaice (Mackie 1982), rainbow trout (Jones 1989) and largemouth bass (Kubitza *et al.* 1997). Betaine had no significant effect in chinook salmon intake when tested as a single taste substance (Hughes 1991, 1993), or in mixtures together with trimethylamine (TMA) and trimethylamine oxide (TMAO) for turbot (Mackie and Adron 1978), TMA-HCl, TMAO and NH_4Cl for jack mackerel (Ikeda *et al.* 1988b), inosine for Atlantic cod (Johnstone and Mackie 1990), TMA-HCl, TMAO-HCl, hypoxanthine, inosine, and IMP and L(+)-lactic acid for European eel (Mackie and Mitchell 1983). Moreover, it was found that omission of betaine, TMA and TMAO increased food acceptance in Atlantic cod (Franco *et al.* 1991). The main ingredient of the commercial product 'Finnstim' (Finnish Sugar Co. Ltd.) is betaine, but at a concentration of 1.5%, it did not act as a stimulant for stellate sturgeon and Siberian sturgeon (Kasumyan *et al.* 1995). Betaine alone at a concentration of 9.7 g of betaine per 1 kg of diet had little effect on freeze-dried diet intake by Dover sole juveniles with a mean weight of 2.5 g, but did have a stimulatory effect on intake for Dover sole with a mean weight of 50 g (Mackie *et al.* 1980). This stimulatory activity of betaine was confirmed for Dover sole (Mackie and Mitchell 1982b). Based on electrophysiological studies, the activity in taste nerves induced by stimulation of betaine was found to vary among fish species (Yoshii *et al.* 1979; Goh and Tamura 1980a; Kiyohara *et al.* 1981; Marui *et al.* 1983a,b; Ishida and Hidaka 1987; Hara *et al.* 1999).

Betaine has important synergistic properties with amino acids or other substances. In plaice, omission of betaine completely abolished activity of nonamino acid components of synthetic squid mixture, although this substance itself was without feeding stimulant activity (Mackie 1982). The palatability of alanine or glycine for red sea bream increased after adding taste-indifferent betaine into the diet (Goh and Tamura 1980a). In extracts of blue crab and flathead grey mullet, a solution containing either betaine alone or the amino acids alone was only 2–9% as effective as the extracts themselves for pigfish and pinfish. Mixtures containing betaine and amino acids were 9–16 times as effective as any of the other components used alone (Carr and Chaney 1976; Carr *et al.* 1977; Carr 1982). Unfortunately, the chemosensory system that mediated fish feeding activity was not defined in this study.

Nucleotides and nucleosides

Inosine, inosine-5′-monophosphate (IMP), 1-methylinosine and inosyl- (3′-5′)-inosine are feeding stimulants for the turbot (Mackie and Adron 1978). Inosine and IMP are feeding stimulants for the brill (Mackie and Mitchell 1985). IMP, but not inosine, was a feed enhancer for juvenile largemouth bass

 © 2003 Blackwell Publishing Ltd,

(Kubitza *et al.* 1997). IMP has also been reported to act as a feeding stimulant for juvenile yellowtail, the effect being potentiated by amino acids (cited by Hosokawa *et al.*; Takeda *et al.* 1984). IMP-Na_2 was highly palatable for jack mackerel; however, other nucleotides tested (adenosine-5′-monophosphate disodium salt, AMP-Na_2; adenosine-5′-diophosphate, ADP-Na_2; adenosine-5′-triphosphate, ATP-Na_2; inosine) were indifferent taste substances (Ikeda *et al.* 1988a). Guanosine-5′-monophosphate (GMP), uridine-5′-monophosphate (UMP), uridine-5′-diphosphate and uridine-5′-triphosphate were also found to be stimulants for jack mackerel; however, other nucleotides, and some of the nucleosides and related compounds (inosine-5′-diphosphate, inosine-5′-triphosphate, guanosine-5′-diphosphate, guanosine-5′-triphosphate, inosine-3′-monophosphate, uridine-3′-monophosphate, 2-deoxy-IMP; allylthio-IMP; xanthosine-5′-monophosphate, adenosine, guanosine, uridine, hypoxanthine) were ineffective (Ikeda *et al.* 1991). The mixture of several nucleotides, AMP-Na, IMP-Na, ADP-Na, ATP-Na_2 and UMP-Na_2, was ineffective as a taste additive for red sea bream (Fuke *et al.* 1981).

The nucleotide fraction of krill synthetic extract was the most effective in increasing the daily feeding rate for marbled rockfish. Most effective among nucleotides were ADP, AMP and IMP; less effective were inosine and UMP and the least effective was ATP (Takaoka *et al.* 1990). The nucleotide fraction of an annelid (Table 2) was inactive or repellent for juvenile Japanese eel. This fraction from the annelid has nearly the same composition as the nucleotide fraction of krill: AMP, UMP-Na_2, IMP-Na_2, ADP-Na_2 and ATP-Na_2 (Takeda *et al.* 1984).

A mixture of the nonamino acid components of synthetic squid extract had high palability for the plaice. However, omission of AMP, lactic acid or TMAO in the mixture greatly decreased the feeding stimulant activity (Mackie 1982).

It is known that the process of conversion of ATP to ADP, AMP and adenosine is the pathway for autolytic degradation of animal flesh, and it has been shown that these substances evoke searching activity and increase the food intake in crustaceans, according to their ranked order of Adenilate Energy Charge (AEC; Zimmer-Faust 1987):

$$\mathrm{AEC} = (\mathrm{ATP} + 0.5\mathrm{ADP})\,(\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP})^{-1}$$

Unfortunately, nothing is known about the relationship between the palatability of fish diet and the value of AEC.

Amines

The adding of TMA to a basal fish diet caused a decrease in the food consumption by chinook salmon fry (2 g in body weight; Hughes 1993), a result similar to that found for first-feeding chinook salmon (Hughes 1991). The same type of response was apparent when TMA or its oxidation product, TMAO, was added to the diet for turbot (Mackie and Adron 1978). In contrast, omission of TMAO greatly reduced the feeding stimulant activity of the nonamino acid compounds for plaice (Mackie 1982).

Trimethylamine oxide, creatine and creatinine were all ineffective as taste additives in food for jack mackerel (Ikeda *et al.* 1988a), and red sea bream showed low taste preference for guanidino compounds – glycocyamine and creatine. The mixture of the organic bases betaine, choline chloride, homarine-HCl, TMAO, TMA-HCl and DMA-HCl hardly induced any feeding activity (Fuke *et al.* 1981). Dimethylthetin, on the other hand, was found to be a taste stimulant for Dover sole; however, various other quaternary amines and related compounds were indifferent taste substances. Examples are dimethylpropiothetin, arsenobetaine, DL-carnitine, trigonelline, homarine, betaine aldehyde and *N,N*-dimethylglycine. TMA, cadaverine and putrescine, products of decomposition of amino acids and quaternary amines, were all inactive as were 2,6-dibromo-phenol and rutin (3′-4′-5,7-tetrahydroxy-flavone-3β-D-rutinosine; Mackie and Mitchell 1982b).

Sugars and other hydrocarbons

Sugars can be stimulants, deterrents or indifferent stimuli for fish. Jones (1990) found that four sugars, D-fructose, sucrose, D-ribose and D-glucose, were palatable for rainbow trout, with some sugars being active only at a concentration of 1.0 M. Two other sugars, D-galactose and D-xylose were indifferent taste substances (Jones 1990). Maltose and glucose were ineffective as taste additives for red sea bream (Fuke *et al.* 1981), and the fraction of Romaine lettuce that contained sugars did not have any effects on the feeding of tilapia (Johnsen and Adams 1986).

Sucrose is a stimulant for different poecilids (for details see section 'Classical taste substances'). Two species belonging to the family Poecilidae – the black molly and the platy – were used to study taste preferences of fish for various sugars and their derivatives. In total, 15 different sugars were tested. It was found that 11 and 5 of these sugars were palatable for platy

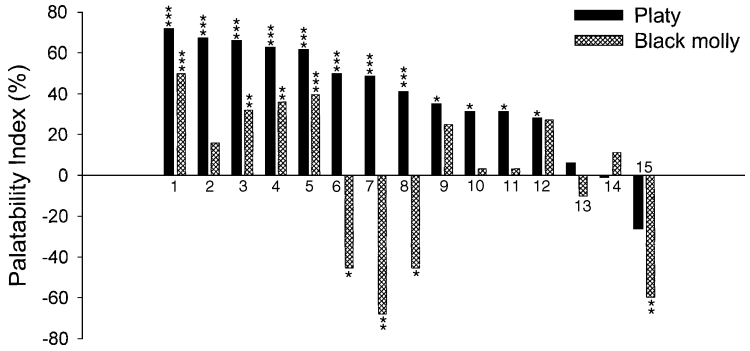

Figure 2 Palatability index for sugars. The palatability indexes for different sugars at 0.1 M for two Poeciliidae fish species, black molly, *Poecilia sphenops* and platy, *Xiphophorus maculatus*. 1, Sucrose; 2, fructose; 3, galactose; 4, saccharin; 5, glucose; 6, arabinose; 7, maltose; 8, lactose; 9, ramnose; 10, sorbose; 11, mannose; 12, sorbitol; 13, mannitol; 14, xylose; 15, ribose. The assessment (Chi-square test) of the palatability of taste substances tested was made in comparison for a blank agar (2%) pellets contained red colour dye, Ponceau 4R (0.05 mM) (Kasumyan, unpublished). In all figures in the review, the significance levels are indicated as follows: $^{***}P < 0.001$; $^{**}P < 0.01$ and $^{*}P < 0.05$.

and black molly, respectively. Among these substances, only four were the same for both the species. Four sugars – arabinose, lactose, ribose and maltose – evoked negative taste responses in black molly; however, none of the sugars tested were deterrents for platy. There was significant correlation between taste preferences for sugars between these two closely related species ($r_s = 0.52$; $P = 0.043$; Fig. 2). In general, the result confirms the conclusion that taste preferences in fish are highly species-specific (for detail see section 'Species specificity').

Organic acids

The succinic, malic and citric acids were ineffective as taste additives for red sea bream (Fuke *et al.* 1981). Rainbow trout reacted positive by ϵ-aminocaproic acid (1.0 M). This acid, as well as L-α-aminobutyric acid and γ-aminobutyric acid, were active at a 10-fold dilution (Jones 1990). Fractions of Romaine lettuce containing malic and citric acids, which are the main constituents in this lettuce, were ineffective taste stimuli for tilapia. Malic acid showed no activity when examined at 0.01 M, but citric acid was a stimulant at this concentration (Johnsen and Adams 1986).

The palatability of 19 organic acids was determined for two cyprinid species, the tench (Kasumyan and Prokopova 2001) and the bitterling (Kasumyan and Prokopova, unpublished). It was found that 17 organic acids were stimulants for tench, the most palatable being malic acid, α-keto-glutaric acid and oxalic acid. Cholic acid was an indifferent taste substance for tench. In bitterling, only cholic acid was a stimulant and 13 other organic acids were deterrent substances. Oxalic acid and α-keto-glutaric acid have the strongest deterrent taste for bitterling (Table 8). The value of Spearman's rank correlation coefficient for these 19 acids was negative and highly significant ($r_s = -0.91$; $P < 0.001$) between these two cyprinid species.

Electrophysiological recordings in common carp showed that the taste responses to monocarboxylic acids reached a peak for compounds having between three and five carbons, whereas dicarboxylic acids gave maximal taste responses for compounds containing five to six carbon atoms (Marui and Caprio 1992). These findings were not matched in behavioural taste responses of tench or bitterling to carboxylic acids, even though these three species are considered to be closely related and belong to the same family (Cyprinidae). In bitterling, for instance, monocarboxylic acids containing between three and five carbon atoms, which include propionic acid, *n*-butyric acid and *n*-valeric acid, were indifferent taste substances, and dicarboxylic acids containing five to six carbons, such as α-keto-glutaric acid and adipic acid, were deterrents. In tench, the palatability level of adipic acid was much less than in oxalic, malonic and succinic acids containing two, three and four carbon atoms, respectively.

Alcohols and aldehydes

There are little data on the palatability of alcohols and aldehydes for fish. Some alcohols such as *n*-hexanol and *n*-octanol were palatable for rainbow trout at a relatively low concentration (10^{-2} and 10^{-3} M), but

© 2003 Blackwell Publishing Ltd,

Table 8 The index of palatability to organic acids at a concentration of 0.1 M for two cyprinid fish, tench (*Tinca tinca*) and bitterling (*Rhodeus sericeus amarus*)

Substances	Tench	Bitterling
Maleic acid	97.2***	−85.1
α-Ketoglutaric acid	97.2***	100***
Oxalic acid	97.1***	100***
Tartaric acid	96.9***	−77.3
Malic acid	96.7***	−86.7
Citric acid	96.6***	−74.7
Malonic acid	96.5***	−88.0
Glycolic acid	95.4***	−38.6
Succinic acid	91.5***	−73.7
Fumaric acid	90.0***	−74.0
Caproic acid	87.9***	−49.4
Adipic acid	88.5***	−58.3
Valeric acid	85.0***	−14.8
Butyric acid	85.0***	0.2
Ascorbic acid	79.4***	−58.3
Formic acid	69.8*	4.0
Propionic acid	69.8*	−19.2
Acetic acid	60.6	2.2
Cholic acid	0	15.5***

in most other compounds tested, the activity was lost. Other alcohols tested such as *n*-propanol and *n*-butanol were indifferent taste substances as were the aldehydes – propionaldehyde and butyraldehyde (Jones 1990).

Other types of substances

It was shown that dimethyl-β-propiothetin (3-dimethyl-3-thiopropanol; DMTP) and dimethyl-thioproprionic acid (2-carboxy-ethyl dimethyl sulphonium bromide; DMPT) are very effective in increasing the number of snaps of test fish directed at suspended food paste. Presence of these substances in food increases growth of red sea bream, yellowtail and Japanese flounder (Nakajima *et al.* 1990). These substances also stimulate feeding and growth of some freshwater fish. Among various sulphur-containing organic compounds, DMTP and, to a lesser extent, dimethylthetin, dipropyl(di)sulphide, dimethylsulfoxide and dimethylsulphone, promoted snapping behaviour in goldfish, crucian carp and common carp. The effect of DMPT was highest at the concentration of 10^{-3} M and decreased when the concentration was different from this value (Nakajima *et al.* 1989a,b; Nakajima 1991). A tertiary sulphonium compound, DMPT, has been shown to be a precursor of dimethyl sulphide (DMS; Challenger and Simpson 1948; Cantoni and Anderson 1956), which generates the odour of the sea in humans (Iida *et al.* 1985; Kasahara and Nishibori 1987). This substance appears to be formed in alga (Iida *et al.* 1985) and higher plants (Larher *et al.* 1977; Van Diggelin *et al.* 1986). Remarkably, levels of DMPT up to 440 mg kg^{-1} and DMS up to 14 mg kg^{-1} were found in various shellfish species (Iida and Tokunaga 1986). The dietary administration of degradative compounds such as DMS, acrylic acid of DMPT, oxidative compounds like dimethyl sulfoxide, dimethyl sulphone, dimethyl sulphite of DMS, various other dialkyl sulphides was much less effective than was DMPT when tested as feed to goldfish. The stimulatory effects of DMPT were much stronger than those of various closely related compounds, which bear shorter or longer methylene and dialkyl carbon chains attached to the sulphur atom compared with that on the DMPT molecule (Nakajima *et al.* 1989b). Both DMPT-enhanced and -unenhanced diets are ingested in almost the same amounts with fish (Nakajima *et al.* 1989a), indicating that the effect of DMPT may be connected with metabolic effects rather than taste attractiveness. There is a brief report that DMT, but not DMP, increases food intake in Dover sole (Mackie and Mitchell 1983).

Mixtures

When stimulants are given together in an experimental pellet or diet, there is often a synergistic effect. This synergism has been shown for pigfish, for which a synthetic mixture of betaine plus 19 amino acids was prepared so that the concentration of each substance was identical to that determined in a shrimp filtrate, and was as stimulatory as the filtrate itself. Both betaine and certain amino acids contributed to the activity of the synthetic mixture. A mixture containing the 19 amino acids alone was only about 28% as effective as the shrimp filtrate, whereas a solution of betaine alone was only 39% as effective as the filtrate (Carr 1976). It is important to note that it is still unclear what chemosensory system – gustation or olfaction – mediates behavioural responses of pigfish to filtrate or mixtures.

Takeda *et al.* (1984) found that uracil-5′-monophophate (5-UMP) enhanced the feeding activity of the Japanese eel to amino acids, while inosine-5′-monophophate (5-IMP) did not. Synergistic effects of 20 free amino acids and 6 nucleotides were found for jack mackerel (Ikeda *et al.* 1988a). A mixture of amino acids alone or a mixture of nucleotides alone had no effect on diet consumption; however, the mixture of

these two main components of synthetic krill extract evoked a positive response. Ikeda and coworkers showed that among 20 free amino acids, tryptophan was the only substance that increased diet consumption. Nevertheless, a diet containing a mixture of all amino acids, including tryptophan, was ineffective. Among six nucleotides, only IMP was a stimulant, whereas a mixture of nucleotides was ineffective. Adding the ineffective nucleotides and other compounds (TMAO, creatine, creatinine or ammonia) into the diet, together with IMP, significantly reduced food consumption (Ikeda *et al.* 1988a,b). It seems plausible that the presence of inactive taste substances together with a stimulant in a mixture may mask the positive effect of a stimulant.

Thresholds

The minimal concentrations needed to give a positive or negative response of a fish towards a given taste stimulus is an important parameter when it comes to flavouring food or bait. Systematic behavioural studies of threshold parameters have been performed for a number of species. For the most common gustatory substances, the threshold concentrations were quite high, but for a few substances, the threshold was relatively low.

The threshold concentration for stimulants is usually around 10^{-2} to 10^{-4} M (Table 9). For common carp, the threshold concentrations for stimulants were 10^{-2} M for L-cysteine, 5×10^{-3} M for citric acid and 0.9 M for calcium chloride (Kasumyan and Morsy 1996). In the brown trout, the threshold concentrations of highly palatable amino acids were in the range of 10^{-2} to 10^{-3} M (Kasumyan and Sidorov 1995c). In the Siberian sturgeon, the threshold concentrations for oral taste were 5×10^{-2} M for citric acid, 9×10^{-2} M for calcium chloride and 1.7 M for sodium chloride (Kasumyan and Kazhlaev 1993a). The threshold for a range of stimulants for the puffer and tilapia was shown to be about 10^{-2} M (Hidaka 1982; Adams *et al.* 1988). In the rainbow trout, a few substances were palatable at a concentration of 10^{-3} M and only L-proline was effective at the concentration 10^{-4} M (Jones 1989, 1990). Quinine, papaverine, sodium ricinoleate and sodium taurocholate, all taste bitter to the human tongue and were found to be feeding deterrents for the Dover sole at a concentration of 5×10^{-6} mol g^{-1} in a moist diet (Mackie and Mitchell 1982b). In the goldfish, quinine-flavoured pellets were rejected at concentrations higher than 10^{-5} M, and caffeine-flavoured pellets were rejected at concentrations higher than $10^{-2.5}$ M (Lamb and Finger 1995). Deoxycorticosterone (21-hydroxypregn-4-ene-3,20-dione) at oral doses of 660 μg (2×10^{-6} M) per pellet caused 94% inhibition in the acceptance of artificial food pellets in sunfish. At the same molar dosage, pregn-4-en-20α-ol-3-ene inhibited food consumption by 58%, while its epimer, pregn-4-en-20β-ol-3-ene did not inhibit feeding significantly (Gerhart *et al.* 1991). The level of deoxycorticosterone in the treated pellets was similar to the levels that can occur in aquatic beetles in the family Dytiscidae (Schildknecht 1971). The minimally active concentration at which stevensine deterred feeding of the bluehead wrasse was between 5.5×10^{-3} and 6.0×10^{-3} M (2.0–2.25 mg mL^{-1}; Wilson *et al.* 1999).

The taste response of red sea bream to alanine at 10^{-3} M concentration was doubtful, but 2×10^{-3} M L-alanine was effective to induce the preference response. The behavioural response to L-alanine increased with increasing stimulus concentrations in the range between 10^{-3} and 10^{-2} M and was saturated at 10^{-2} M (Goh and Tamura 1980a). In tench, the acceptance ratio of pellets containing malic acid increased six times in the range between 10^{-2} and 10^{-1} M. The dose–response relationships for the acceptance ratio for two fish species are presented in Fig. 3. The retention time for keeping the pellets in the mouth increased with concentration, an example of which is shown in Fig. 4.

For a single species, namely tench, the taste threshold has been found to vary with the method used to assess the palatability; thus acceptance ratio, number of snaps at pellets, retention time of the pellet at first snap and total retention time during a trial give different threshold values (Table 10).

Based on threshold concentrations determined by behavioural assays, it is possible to estimate the amount of substance in one pellet, which is sufficient to release a significant taste response. These amounts were 4.27 and 3.39 μg for L-cysteine and citric acid, respectively (common carp), 0.236 μg for L-alanine (European minnow) and 0.353 μg for L-aspartic acid (frolich char). These values correspond to 10^{-9} to 10^{-10} mole or 10^{15}–10^{16} molecules per pellet. However, all substances except those that are situated in the surface layer in a pellet do not stimulate the gustatory receptors. The substance part inside the pellet is unavailable to taste receptors. Therefore, the actual quantities of the substance sufficient for eliciting a significant taste response would be lower than calculated by one to two orders of magnitude.

© 2003 Blackwell Publishing Ltd,

Table 9 Taste thresholds

Substances	Threshold concentration (mM)	Species	Source
Incitants			
Citric acid	5	Acipenser baerii	Kasumyan and Kazhlaev (1993b)
	5	*Acipenser stellatus*	Kasumyan and Kazhlaev (1993b)
Suppressants			
Calcium chloride	90	*Acipenser baerii*	Kasumyan and Kazhlaev (1993b)
	90	*Acipenser stellatus*	Kasumyan and Kazhlaev (1993b)
Stimulants			
L-Alanine	1	*Phoxinus phoxinus*	Kasumyan, unpublished
	2	*Chrysophrys* (*Pagrus) major*	Goh and Tamura 1980
	100	*Fugu pardalis*	Hidaka (1982)
L-Aspartic acid	1	*Salvelinus alpinus erhytrinus*	Kasumyan and Sidorov, unpublished
	10	*Salmo trutta caspius*	Kasumyan and Sidorov (1995)
Betaine	10	*Fugu pardalis*	Hidaka (1982)
L-Cysteine	5	*Salvelinus alpinus erhytrinus*	Kasumyan and Sidorov, unpublished
	10	*Cyprinus carpio*	Kasumyan and Morsy (1996)
	25	*Tinca tinca*	Kasumyan and Prokopova (2001)
L-Glutamic acid	5	*Salvelinus alpinus erhytrinus*	Kasumyan and Sidorov, unpublished
L-Glutamine	10	*Phoxinus phoxinus*	Kasumyan, unpublished
Glycine	100	*Fugu pardalis*	Hidaka (1982)
L-Leucine	10	*Oncorhynchus mykiss*	Jones (1989)
L-Phenylalanine	10	*Salmo trutta caspius*	Kasumyan and Sidorov (1995)
	10	*Oncorhynchus mykiss*	Jones (1989)
L-Proline	0.1	*Oncorhynchus mykiss*	Jones (1989)
	10	*Phoxinus phoxinus*	Kasumyan, unpublished
	10	*Fugu pardalis*	Hidaka (1982)
L-Serine	10	*Fugu pardalis*	Hidaka (1982)
L-Tyrosine	0.1	*Xiphophorus maculatus*	Kasumyan, unpublished
	1	*Salmo trutta caspius*	Kasumyan and Sidorov (1995)
L-Valine	10	*Phoxinus phoxinus*	Kasumyan, unpublished
Citric acid	5	*Cyprinus carpio*	Kasumyan and Morsy (1996)
	1.0–10	*Tilapia zillii*	Adams *et al.* (1988)
	10	*Thymallus thymallus*	Kasumyan and Sidorov, unpublished
	50	*Acipenser baerii*	Kasumyan and Kazhlaev (1993b)
	50	*Acipenser stellatus*	Kasumyan and Kazhlaev (1993b)
Calcium chloride	90	*Acipenser baerii*	Kasumyan and Kazhlaev (1993b)
	90	*Acipenser stellatus*	Kasumyan and Kazhlaev (1993b)
	900	*Cyprinus carpio*	Kasumyan and Morsy (1996)
Sodium chloride	1700	*Acipenser baerii*	Kasumyan and Kazhlaev (1993b)
Sucrose	3	*Ctenopharyngodon idella*	Kasumyan, unpublished
Xylose	10	*Leuciscus leuciscus*	Kasumyan and Fokina, unpublished
Succinic acid	10	*Thymallus thymallus*	Kasumyan, unpublished
α-Ketoglutaric acid	10	*Thymallus thymallus*	Kasumyan, unpublished
Maleic acid	25	*Tinca tinca*	Kasumyan and Prokopova (2001)
IMP	2×10^{-6} [a]	*Trachurus japonicus*	Ikeda *et al.* (1991)
Inosine	6×10^{-8} [a]	*Scophthalmus maximus*	Mackie and Adron (1978)
Deterrents			
Quinine	0.1	*Carassius auratus*	Lamb and Finger (1995)
Caffeine	50	*Carassius auratus*	Lamb and Finger (1995)
Stevensine	5.5–6.0	*Thalassoma bifasciatum*	Wilson *et al.* (1999)

The threshold to different types of taste substances in fish. Values marked by an superscript letter are in mM g^{-1} dry diet.

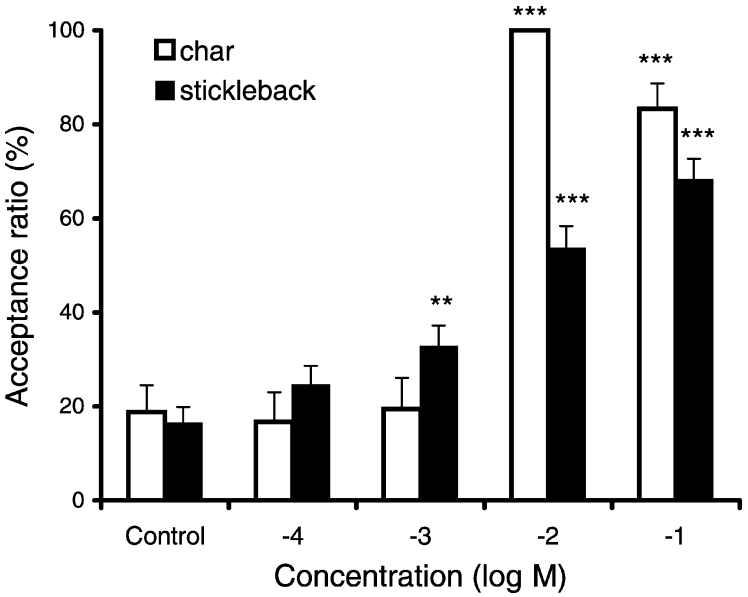

Figure 3 Effect of concentration on acceptance ratio. Dose–response relationship for acceptance ratio of L-cysteine in Frolich char, *Salvelinus alpinus erythrinus*, juveniles and for acceptance ratio of L-Glutamine in nine-spined stickleback, *Pungitius pungitius* (Gasterosteidae). Control – blank pellets. Vertical columns and T-bars are the mean and the SEM (Kasumyan, Sidorov and Fokina, unpublished).

The threshold concentrations for substances determined using bioassays are much higher than the thresholds determined by electrophysiological recordings from the teleost fish. Recordings from the facial, glossopharyngeal or vagal taste nerves revealed threshold concentrations between 10^{-7} and 10^{-9} M for free amino acids and some other substances (Konishi and Zotterman 1961; Kaku *et al.* 1980; Kiyohara *et al.* 1981; Davenport and Caprio 1982; Kanwal and Caprio 1983; Marui *et al.* 1983a,b; Marui and Caprio 1992; Hara *et al.* 1999). There might be several reasons for the discrepancy between the threshold concentrations obtained by behavioural and electrophysiological methods. It was supposed that the discrepancy might be related to the methods used to stimulate the taste receptors (Kasumyan and Sidorov 1995c; Kasumyan and Morsy 1996). In the electrophysiological studies, a relatively larger area with taste receptors in the fish mouth or barbel is irrigated by the test solution. In the behavioural assay, a limited number of taste receptors are stimulated because normally the pellet is relatively small – 2.5 mm in length and 1.5 mm in diameter. Further, in the electrophysiological studies, the solution is applied for a long period (5 s; Hara *et al.* 1999). In behavioural assays, the time that a fish spends in making a decision about the taste of the food item and consequently either swallowing or rejecting it, is generally shorter than in the electrophysiological experiments (Kasumyan and Sidorov 1993a,b, 1995c).

The discrepancy between the threshold concentrations obtained in the behavioural and electrophysiological experiments could be associated with peculiarities of functional relationship between the taste and the mechanosensory system. In behavioural assays, both the chemoreceptors and the mechanoreceptors are stimulated simultaneously. This aspect might be of significance and there might be a functional interaction between the gustatory

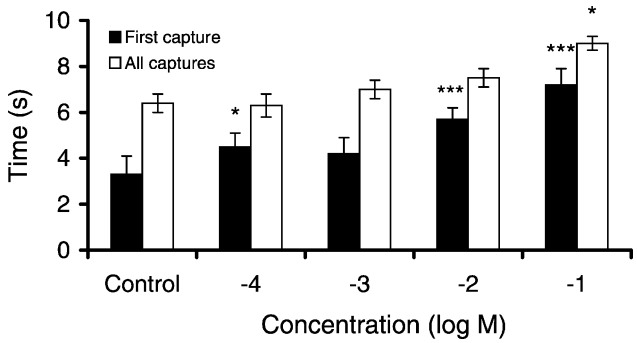

Figure 4 Effect of concentration on pellet retention time. Dose–response relationship for pellet retention time contained L-glutamine in nine-spined stickleback *Pungitius pungitius*. Control – blank pellets. Vertical columns and T-bars are the mean and the SEM (Kasumyan and Fokina, unpublished).

© 2003 Blackwell Publishing Ltd,

Table 10 Taste responses to different concentrations

Substance	Concentration (mM)	Acceptance ratio (%)	Mean number of snaps	Mean retention time (s)		Number of trials
				First capture	All captures	
Maleic acid	100	90.7***	1.1***	19.8***	20.2***	150
	50	53.3***	1.3***	13.3***	14.9***	150
	10	14.7***	1.3***	5.3***	6.1**	204
	1	3.7	1.4**	2.2	3.3	54
	0	2.2	2.6	2.3	4.1	225
L-Cysteine	100	90.1***	1.2***	18.6***	19.7***	91
	50	61.5***	1.5	12.8***	14.8***	91
	25	37.8***	1.5	6.3***	7.3***	90
	1	7.8	1.6	3.1*	4.1*	115
	0.1	7.9	1.6	2.3	3.2	76
	0	4.0	1.5	2.1	2.9	75

Behavioural taste response of tench, *Tinca tinca* for agar pellets containing maleic acid or L-cysteine in different concentrations (modified from Kasumyan and Prokopova 2001).

and the mechanosensory systems. It has been suggested that fish taste buds (basal cells) are mechanosensitive (Jakubowski 1983; Reutter 1986; Reutter 1987; Reutter 1992). The taste and tactile inputs are topographically closely connected in gustatory centres located in the medulla oblongata. In channel catfish, the facial gustatory centre, a facial lobe, is organised into lobules for representation of taste and tactile inputs from the barbels, lips, snout and flank. In cyprinids such as goldfish, the centre for intraoral taste, the vagal lobe, is organised anatomically into layers. This structure contains a representation of taste and tactile inputs from the palatal organ in the oral cavity (Morita and Finger 1985; Kanwal and Caprio 1988; Marui *et al.* 1988; Hayama and Caprio 1990; Kanwal and Finger 1992). There is evidence that chemo- and mechanosensory systems are tightly knit in the oral cavity. In bottom-feeder cyprinid fish, the local contraction of the palatal organ musculature, which is entirely dependent upon the local characteristics of the gustatory stimulation, traps the desirable food particles between the palatal organ and the floor of the oral cavity. Once the food particles have been trapped, the waste is flushed out from the oral cavity and subsequently, the food particles are swallowed (Sibbing *et al.* 1986; Osse *et al.* 1997). In sturgeon, the food particles containing incitants were snapped at after preliminary touching of food items by barbels covered by external taste buds (Kasumyan and Kazhlaev 1993a).

It might be suggested that the stimulation of mechanoreceptors associated with the first touch by a food particle of taste buds is essential for mediation of the behavioural taste response. In other words, it might be that the mechanosensory system facilitates the transmission of taste information to the motor centres in the fish brain. It is also conceivable that the mechanosensory system can desensitise this pathway, if not activate it. This hypothesis is supported by the observation that some anosmic fish loose their ability to react even at high concentrations of food extract, in spite of an intact taste system (Kasumyan and Ponomarev 1986, 1989; Kasumyan and Kazhlaev 1993b; Kasumyan and Devitsina 1997), although the anosmic catfish of the genus *Ictalurus* does not lose its ability to find food (Bardach *et al.* 1967).

Specificity of taste preferences

Species specificity

It has been suggested that taste stimulants are similar within a fish family (Mackie and Mitchell 1985). This conclusion is based on the results obtained for only a few fish species and can therefore be considered preliminary in nature. During the last decade, there has been significant progress in the field of taste preferences of fish. A number of different species have been tested and more extensive methods have been used; thus the basis for the classification of fish species by their taste preferences and their relationships to fish taxonomy has increased. The number of species studied is still narrow; however, a comparative analysis of these scanty data is possible because the

taste preferences for the majority of species were based upon similar methods, using the same substances and the same carrier.

Comparative analyses of results obtained for more than 20 fish species reveal that oral taste preferences are highly species-specific. The differences among fish species are apparent at a comparison of the width of spectra for stimulants and deterrents. The results presented in Table 6 demonstrate that few of the 21 amino acids tested are effective taste stimuli for chub, stellate sturgeon, guppy and frolich char. In Arctic flounder and crucian carp, all free amino acids were indifferent. In grass carp and platy, many amino acids were either stimulants or deterrents. The ratio between the number of stimulant amino acid to the number of deterrent amino acids is 3 : 17 in grass carp and 13 : 2 in platy (Kasumyan and Sidorov 1995d; Kasumyan and Nikolaeva 1997, 2002; Kasumyan 1999a).

The spectra of stimulant and deterrent amino acids differ not only in the width but also in composition. In many cases, the same amino acid evokes dramatically different oral taste response in fish, including closely related species from the same family or genus. L-Valine, L-serine, L-threonine, L-arginine, L-glutamine and L-proline cause opposite taste responses in common carp versus wild goldfish; L-cysteine, L-methionine, L-valine, L-isoleucine and L-serine in chum salmon versus brown trout; L-arginine, L-asparagine, L-histidine, L-methionine, L-proline, L-leucine and L-isoleucine in black molly versus platy; L-alanine in stellate sturgeon versus Siberian sturgeon (genus *Acipenser*) and L-cysteine, L-lysine and L-glutamic acid in black molly versus guppy (genus *Poecilia*).

The number of amino acids that evoke the same taste response, is much smaller in the species mentioned above. Only L-methionine and L-phenylalanine evoke the same taste responses in both common carp and wild goldfish; L-histidine, L-phenylalanine and L-tyrosine in chum salmon and brown trout and L-alanine, L-cysteine, L-phenylalanine, L-serine, L-threonine and L-tyrosine in black molly and platy. There is no single amino acid that evokes the same oral taste response in stellate sturgeon, Siberian sturgeon and Russian sturgeon, all of which belong to the same genus *Acipenser* (Table 7).

The responses to classical taste substances differ among the fish species tested (Table 6). Citric acid was a stimulant for the salmonids brown trout, brook char, lake char and frolich char, but a deterrent for chum salmon. Among cyprinids, citric acid is a stimulant for common carp, grass carp and tench, but a deterrent for crucian carp, roach and bitterling. Sodium chloride is a stimulant for tench, roach and dace, but a deterrent for wild goldfish. Calcium chloride is a stimulant for common carp, chub and tench, but a deterrent for wild goldfish (Table 4).

There are examples of similarities in taste responses in closely related fish species. This is more evident among classical taste substances than among free amino acids. For instance, all four classical taste substances possess the same taste properties for Siberian sturgeon and stellate sturgeon (genus *Acipenser*). Sucrose was a stimulant for three Poeciliidae species tested. Citric acid was a stimulant for lake char, brook char and frolich char and it was a deterrent for crucian carp and wild goldfish. Calcium chloride was a deterrent for brook char and lake char, and was an indifferent stimuli for frolich char (Table 4).

The palatability of substances is different in sympatric fish that inhabit the same water, and have the same biotopes and similar feeding ecology and feeding behaviours. For example, the taste preferences for a number of amino acids did not coincide amongst European minnow, dace and chub. The experimental specimens of these fish were caught in the early autumn of their first year of life in the same stretch of a small stream, and were used for bioassay after being kept for 10 months together in a general aquarium and being fed with the same feeds. The palatability of numerous substances was different in common carp, crucian carp, wild goldfish and tench, fish that inhabit the same water and have similar diets (Kasumyan and Morsy 1996; Kasumyan and Prokopova 2001; Kasumyan, unpublished observation).

The differences in taste preferences are confirmed by the correlation analysis of amino acid palatability for 21 fish species tested (Table 11). A positive correlation was found for 10 (4.8%) out of 210 possible pairs, a negative correlation was found for six pairs only (2.9%). All these pairs had a low level of relationships. Eight fish species did not show any significant correlation with any other species, and 13 fish species had a significant relationship with one to four other species. In most cases, the positive relationship was between species that are distant in systematic position, ecology and feeding type. These pairs were: common carp–guppy, guppy–grass carp, black molly–navaga, navaga–chum salmon and chum salmon–platy. A positive correlation was not found in related species such as guppy, black molly and platy, all belonging to the same Poecilidae family and the

© 2003 Blackwell Publishing Ltd,

same genus as crucian carp and wild goldfish, or Russian sturgeon, Siberian sturgeon and stellate sturgeon (genus *Acipenser*). Moreover, correlation between taste responses to free amino acids was negative in the pair guppy–platy. As seen from Table 11, the number of positive correlations in tasts preferences between closely related fish species is very limited and seems to be an exception rather than the rule. For instance, a positive correlation was found in only 4 cases among the 10 cyprinid fish species tested, and in one case between three salmonid fish species. One positive correlation was found between acipenserids and between poeciliids.

Thus, taste preferences are characterised by strong species specificity. These data clearly demonstrate an important and undoubtedly leading role of gustatory reception in the feeding selectivity in fish, and in their capacity to consume appropriate food items that are specific to them.

Considerable species differences in the range of amino acids that are stimulatory have been revealed by electrophysiological studies (for review, see Marui and Caprio 1992).

Population specificity

Brown trout is a polytypic fish species, which has developed different geographically isolated subspecies over a vast area. Fish from three widely separated catchments were used to compare taste preferences in specimens belonging to different populations. Brown trout juveniles (5–6 cm in body length) were obtained from Terek River (Caspian Sea basin), Luga River (Baltic Sea basin, the Gulf of Finland) and Vorobejv stream (White Sea basin, Kandalaksha Bay). Fish were delivered to the Moscow State University and were used for trials after being kept for 1–2 months in a common aquarium. The trials were performed in different years with fish delivered from different water basins. Four classical taste substances and 21 common free amino acids were used as taste stimuli (Kasumyan and Sidorov 1995a).

It was found that taste preferences were similar in brown trout juveniles from all three populations (Fig. 5). Citric acid and L-cysteine were the most palatable substances for Caspian Sea, Baltic Sea and White Sea brown trout. Fish in all trials swallowed agar pellets containing these substances after the first snap. The retention time correlated positively with the acceptance ratio of pellets. L-Isoleucine, L-glutamine, L-valine and glycine were the strongest deterrent taste stimuli, while sodium chloride, calcium chloride, sucrose and many of the free amino acids were indifferent taste substances (Kasumyan and Sidorov 1995a,c). A positive correlation was found between fish in the taste preferences for 25 substances (21 free amino acids and 4 classical taste substances; Table 12).

Caspian brown trout belong to the 'Danube' phylogenetic group of *Salmo trutta* populations, and Baltic Sea brown trout and White Sea brown trout belong to the 'Atlantic' phylogenetic group. These groups of brown trout populations were separated at least 500 000 years ago, according to a genetic study (Osinov and Bernache 1996; Bernache 2001). The most recent connection between the Caspian Sea basin and both the Baltic Sea and White Sea basins has occurred during the last glacial period (12 000–15 000 years ago). There have been many thousands of generations of brown trout since this time, but still, taste preferences remain similar. The results indicate that taste preferences do not have impressive interpopulation specificity in fish. It has been shown for humans that persons of different nationalities have similar taste preferences for chemical substances.

Specificity among conspecifics

Given the similarity between different fish population of the same species, it is noteworthy that there is variability in taste preferences between individuals of a population. Individual variation in diet has been found in fish that inhabit the same natural water body, but also in fish maintained in experimental conditions (Bryan and Larkin 1972; Ringler 1985; Bridcut and Giller 1995). It was noted that taste preferences at the individual level might vary dramatically among conspecifics. In rainbow trout, the number of specimens with distinctive taste preferences is about 1 per 25 fish (Jones 1989) or 3 per 11 fish (Mearns *et al.* 1987). The particular specimens used by these authors either consistently rejected both the blank and the shrimp-flavoured pellets or, conversely, swallowed both of them.

A study of individual variability of taste preferences was performed on the common carp (Kasumyan 2000). Oral taste responses were assessed for twenty-four 1-year-old juveniles. Amino acids L-cysteine (0.1 M) and L-glutamic acid (0.01 M) were used as taste stimuli, and blank pellets were used as the control. It was found that the average acceptance ratio was 97.3% for L-cysteine and 41.8% for L-glutamic acid. The acceptance ratio for blank pellets was

Table 11 Correlation of taste preferences between species

PSpecies:	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1 – Brown trout, *Salmo trutta caspius*	0.63**	-0.26	-0.18	-0.01	0.11	-0.10	0.44*	0.14	0.39	0.19	-0.07	-0.24	-0.46*	-0.30	-0.60**	-0.07	0.02	-0.01	-0.37	-0.33
2 – Frolich char, *Salvelinus alpinus erythrinus*	-	0.03	-0.04	0.05	-0.01	0.16	0.27	0.02	0.43	0.38	-0.09	-0.30	-0.08	-0.16	-0.26	0.02	-0.21	0.26	-0.12	-0.06
3 – Chum salmon, *Oncorhynchus keta*		-	0.36	-0.44*	0.44*	-0.19	-0.59**	-0.05	-0.25	0.30	0.11	0.02	0.07	-0.09	0.22	-0.25	0.06	-0.23	0.44*	0.18
4 – Black molly, *Poecilia sphenops*			-	-0.17	0.10	-0.20	-0.08	-0.002	-0.23	-0.12	-0.03	0.36	0.33	0.29	0.28	-0.13	0.16	0.001	0.60**	0.04
5 - Guppy, *Poecilia reticulata*				-	-0.58**	-0.04	0.25	-0.13	0.50*	-0.06	-0.19	-0.18	0.29	0.19	0.31	0.21	-0.19	0.52*	-0.29	-0.25
6 - Platy, *Xiphophorus maculatus*					-	-0.22	-0.38	0.13	-0.003	0.38	0.19	0.21	-0.17	-0.16	-0.02	-0.21	0.06	-0.46**	0.28	0.31
7 – Siberian sturgeon, *Acipenser baerii*						-	0.34	-0.09	-0.34	0.03	0.33	-0.05	0.09	-0.22	-0.27	-0.30	-0.34	-0.000	-0.16	0.29
8 – Russian sturgeon, *Acipenser gueldenstaedtii*							-	0.15	-0.01	-0.12	0.003	-0.03	-0.01	-0.004	-0.36	0.14	-0.17	-0.05	-0.19	-0.01
9 – Stellate sturgeon, *Acipenser stellatus*								-	0.13	-0.37	0.07	-0.05	-0.08	0.02	-0.14	0.22	-0.06	-0.07	0.26	0.05
10 – Common carp, *Cyprinus carpio*									-	0.20	-0.16	-0.40	-0.13	0.01	0.07	0.29	-0.09	0.41	-0.36	-0.26
11 – Crucian carp, *Carassius carassius*										-	0.14	-0.23	-0.22	-0.43	-0.13	-0.41	-0.01	-0.25	-0.003	-0.03
12 – Goldfish, *Carassius auratus*											-	0.26	0.04	-0.17	-0.07	-0.30	0.17	0.07	-0.22	0.07
13 – Roach, *Rutilus rutilus*												-	0.26	0.41	0.25	0.11	0.42	-0.02	0.26	0.18
14 – Dace, *Leuciscus leuciscus*													-	0.61**	0.58**	0.34	-0.16	0.27	0.21	0.25
15 – Chub, *Leuciscus cephalus*														-	0.69**	0.62**	0.04	0.14	0.13	0.22
16 – European Minnow, *Phoxinus phoxinus*															-	0.29	-0.05	0.18	0.37	0.24
17 – Tench, *Tinca tinca*																-	-0.03	0.34	-0.10	0.03
18 – Bitterling, *Rhodeus sericeus* ***amarus***																	-	-0.05	0.12	-0.42
19 – Grass carp, *Ctenopharyngodon idella*																		-	-0.33	-0.02
20 – Navaga, *Eleginus navaga*																			-	0.19
21 – Arctic flounder, *Liopsetta glacialis*																				-

The values of Spearman rank correlation coefficient for taste preferences to 21 free amino acids (19 for Siberian sturgeon) between different fish species. Numbers in frames represent correlation coefficients for species of the same family. Salmonidae 1–3, Poecilidae 4–6, Acipenseridae 7–9, Cyprinidae 10–19.

© 2003 Blackwell Publishing Ltd.

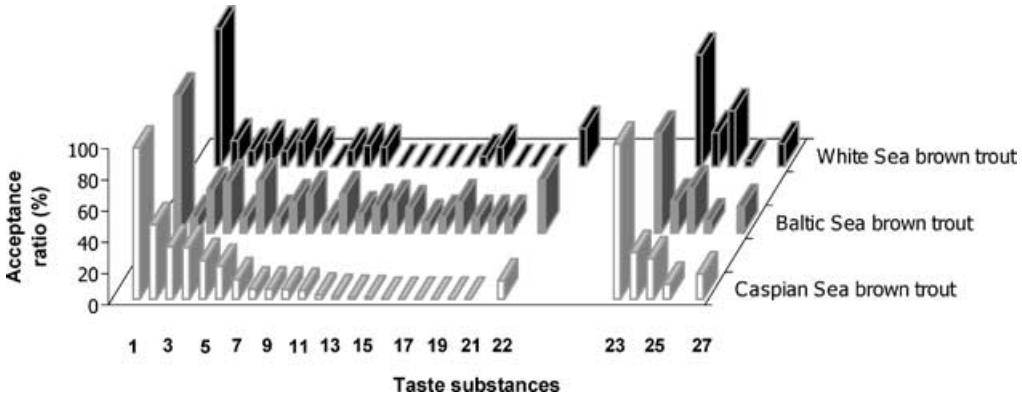

Figure 5 Taste preferences to free amino acids and classical taste substances in brown trout. Taste preferences in juveniles of brown trout (*Salmo trutta*) from different sea basins (modified from Kasumyan and Sidorov 1995). 1 – L-Cysteine, 0.1 M; 2 – L-tyrosine, 0.001 M; 3 – L-tryptophan, 0.01 M; 4 – L-aspartic acid, 0.01 M; 5 – L-histidine, 0.1 M; 6 – L-phenylalanine, 0.1 M; 7 – L-arginine, 0.1 M; 8 – L-glutamic acid, 0.01 M; 9 – L-lysine, 0.1 M; 10 – L-proline, 0.1 M; 11 – L-alanine, 0.1 M; 12 – L-leucine, 0.1 M; 13 – L-threonine, 0.1 M; 14 – L-asparagine, 0.1 M; 15 – L-serine, 0.1 M; 16 – glycine, 0.1 M; 17 –L-Norvaline, 0.1 M; 18 – L-methionine, 0.1 M; 19 – L-valine, 0.1 M; 20 – L-isoleucine, 0.01 M; 21 – L-glutamine, 0.1 M; 23 – citric acid, 0.26 M; 24 – calcium chloride, 1.73 M; 25 – sodium chloride, 0.9 M; 26 – sucrose, 0.29 M; 22 and 27 – respective controls (blank pellets; Kasumyan and Sidorov, unpublished).

17.0%. The value of the coefficient of variation for the acceptance ratio was low for highly palatable L-cysteine (CV = 5.14%), while it was much higher for less palatable L-glutamic acid (56.4%), and especially for blank pellets (89.0%). All specimens tested showed a positive taste response to L-cysteine, with seven specimens showing positive taste responses to L-glutamic acid. L-glutamic acid was found to be a deterrent for one specimen among the 24 carps tested. The fish rejected flavoured pellets significantly more often than the blank pellets. Many of the fish preferred pellets flavoured by L-cysteine and showed an indifferent response for pellets containing L-glutamic acid. There were several specimens with a relatively low taste preference for L-cysteine and a very high taste preference for L-glutamic acid.

Table 12 Comparing populations

Origin of population	Baltic Sea		White Sea	
	FAA	FAA + CTS	FAA	FAA + CTS
Caspian Sea	0.49*	0.55**	0.68**	0.76***
Baltic Sea	–	–	0.47*	0.60**

The values of Spearman's rank correlation coefficient for taste preferences to free amino acids (FAA) and classical taste substances (CTS) between three populations of brown trout (*Salmo trutta*) juveniles from Caspian Sea, Baltic Sea and White Sea basins. See text for details.

Statistical analysis showed that three specimens had an acceptance ratio for L-glutamic acid that was more than 1 SD from the mean value for the 24 fish tested. Nine specimens showed a similar deviation from the mean in their reaction to blank pellets.

These results show an interesting aspect of taste preferences. Taste responses are more stable and invariable for highly palatable substances than for substances with low level of palatability. This result might explain the food specialisation between conspecifics, which is often found among wild fish populations (Bryan and Larkin 1972; Ringler 1985; Bridcut and Giller 1995) and the disproportional artificial food intake in cultured stocks (Storebakken and Austreng 1988). Individual variability in taste preferences has been found in other groups of animals (Stevens 1990) and also in humans (Prescott and Stevenson 1995). The source of individual differences is still poorly understood, and it has been suggested that these individual patterns of taste preferences are genetically determined (Kasumyan 2000). The individual variability can be used in aquaculture to promote strains that have particular taste preferences.

Specificity between sexes

A study of sex variability in taste preferences of fish was performed on adult guppy (Nikolaeva and Kasumyan 2000). Citric acid, sucrose, calcium chloride, glycine, L-glutamic acid and L-histidine were

used as taste substances. It was found that the males and females of guppy have similar taste preferences for the substances tested. Sucrose, glycine and L-glutamic acid were stimulants for both male and female. Citric acid, sodium chloride, calcium chloride and L-histidine had an indifferent taste for fish. The value of the Spearman's rank correlation coefficient was 0.97 ($P < 0.01$) for acceptance ratio between specimens of different sexes. The acceptance ratio for every substance was higher in females than in males (Fig. 6).

The results indicate that there are no differences between male and female guppy in taste preferences. The revealed coincidence of taste preferences agrees well with the data on the feeding of guppy in natural water bodies. It was shown that females and males use the same groups of food objects, which include green algae and diatoms, benthos, detritus and larvae of insects, as food (Dussault and Kramer 1981).

Sex dimorphism with respect to taste preferences is not a characteristic attribute in fish; however, the guppy does show sex differences in its behavioural taste response pattern. The intensity of some of the elements in the taste response like retention time of pellets in the mouth and number of snaps at pellets was lower in males than in females. In total, the duration of the taste response was shorter among males than among females. Observations carried out on guppy show that males spent their time courting rather than feeding (Farr and Herrnkind 1974; Dussault and Kramer 1981), which indicates that the male behaviour strategy is orientated towards reproduction, with nonreproductive behaviour being minimised.

Genetics of taste preferences

It seems obvious that taste preferences in fish as in other vertebrates are genetically determined. However, only one study has been performed with the purpose of investigating the genetic nature of the food preferences in fish (Kasumyan and Nikolaeva, unpublished study). This study was performed by comparing taste preferences in wild female goldfish, male common carp and hybrids between these two cyprinid species. There were two main reasons to use these species – the palatability for some substances is different in these two species, and goldfish and common carp readily yield hybrids. All specimens used for these experiments were reared on chironomid larvae and were maintained in water with similar temperature, oxygen supply and light cycle.

It was found that the taste preferences for the classical taste substances were similar in carp and hybrids. However, the rank according to the preference of these substances was significantly different between hybrids and wild goldfish (Fig. 7). Responses of the hybrids were particular in that none of the amino acids had any effect on pellet consumption, indicating that none of the amino acids were stimulants. This is a striking observation because six and eight amino acids are stimulants for carp and wild goldfish, respectively. There was a positive correlation between the palatability of all substances tested, which include four classical taste substances and 21 free amino acids for carp and hybrid ($r_s = 0.53$; $P < 0.01$). However, no significant relationship existed between wild goldfish and hybrids ($r_s = -0.01$;

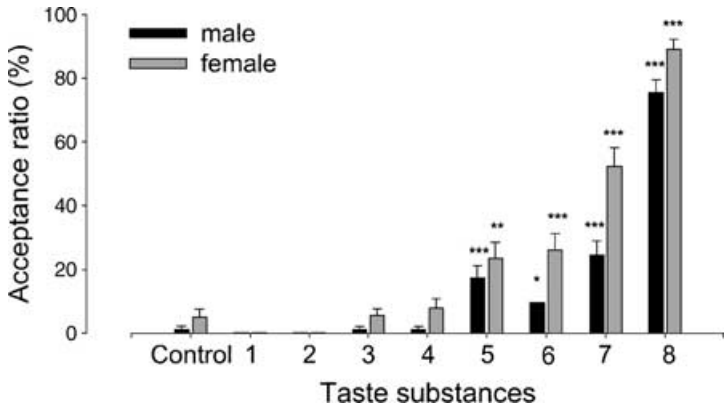

Figure 6 Taste preferences in males and females. Taste preferences in male and female of guppy (*Poecilia reticulata*) to classical taste substances and for some of the free amino acids (modified from Nikolaeva and Kasumyan 2000). 1 – Sodium chloride, 0.9 M; 2 – calcium chloride, 1.73 M; 3 – citric acid, 0.26 M; 4 – L-histidine, 0.1 M; 5 – sucrose, 0.29 M; 6 – L-glutamic acid, 0.01 M; 7 – glycine, 0.1 M; 8 – *Chironomidae* larvae water extract, 75 g(wet weight) L^{-1}. Control – blank pellets. Vertical columns and T-bars are the mean and the SEM (Nikolaeva and Kasumyan 2000).

 © 2003 Blackwell Publishing Ltd,

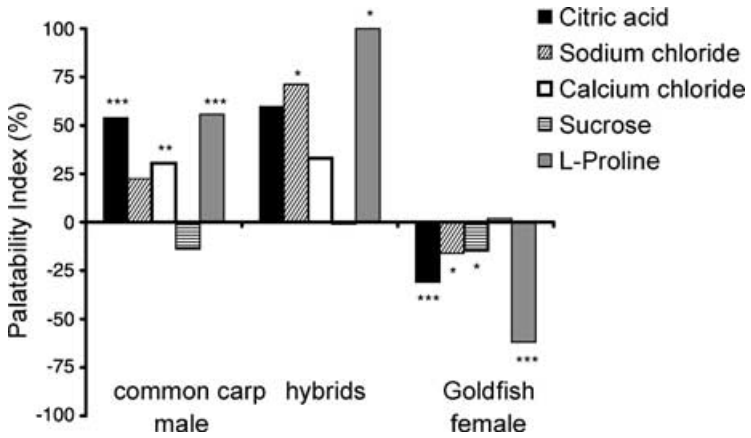

Figure 7 Genetics of taste preferences. Palatability index for classical taste substances and L-proline in common carp (*Cyprinus carpio*), wild goldfish (*Carassius auratus*) and hybrids between these two species. 1 – Citric acid, 0.26 M; 2 – sodium chloride, 0.9 M; 3 – calcium chloride, 1.73 M; 4 – sucrose, 0.29 M; 5–L-proline, 0.1 M (Kasumyan and Nikolaeva, unpublished).The assessment (Chi-square test) of the palatability of taste substances tested was made in comparison for a blank agar (2%) pellet containing red colour dye, Ponceau 4R (0.05 mM).

$P > 0.05$). This study shows that the taste preferences are determined genetically and seem to be patroclinous.

A recent study on different strains of rainbow trout investigated the gustatory responses from the palatine nerve to 10 substances, mainly amino acids. The results indicated that the strains could be divided into two response groups: one group including the Kamloops, Manx, Mount Lassen, Sunndalsøra, Tagwerker and Victoria strains, and the second group including the Nisqually, Nikko and steelhead strains. It was concluded that the functional variability of the gustatory system may be of genetic origin (Hara *et al.* 1999).

Ontogeny of taste preferences

The development of taste preferences is another aspect of fish chemoreception that has been scantily explored. Most studies concerning ontogeny of the gustatory system in fish have been focused on the morphology of the taste buds. Several studies have determined the stage at which taste buds appear in the mouth or on the body surface (Appelbaum *et al.* 1983; Pevzner 1985; Kawamura and Washiyama 1989; Devitsina and Kazhlaev 1993; Hansen *et al.* 2002). According to these studies, the taste buds in fish appear 1–2 days or even only hours before fish larvae start to catch food items, which is at the beginning of exogenous feeding.

It has been found that there is good correspondence between the development of the gustatory system and the ability to discriminate taste properties of food items. Experiments performed on larvae and juveniles of Siberian sturgeon and stellate sturgeon have shown that, up to the time when it becomes necessary to estimate the gustatory qualities of food, fish have a well-expressed ability to discriminate taste stimuli. Sturgeon larvae of 'start feed' age show the same oral and extraoral taste behavioural responses to the classical taste substances represented by sucrose, sodium chloride and calcium chloride as do the older juveniles. Citric acid was an indifferent substance for sturgeon larvae, but evoked conspicuous oral and extraoral taste responses in sturgeon juveniles. Citric acid was a strong incitant for sturgeon juveniles and induced a high capture rate of pellets. Nearly all pellets flavoured with citric acid and touched by a fish barbel were captured, but they were immediately rejected (Kasumyan 1992; Kasumyan and Kazhlaev 1993a).

The conclusion that larvae do not respond to amino acids, which are taste stimuli for older juveniles, has been confirmed by the comparison of extraoral and oral taste responses of Russian sturgeon (Kasumyan *et al.* 1992). A smaller number of substances acted as incitants and stimulants in larvae (21–25 mm body length) than in juveniles (60–70 mm body length). For larvae, 11 and 1 amino acids were incitants and a stimulant, respectively, and for juveniles, 16 and 6 amino acids were incitants and stimulants, respectively (Table 13). There was a positive correlation between extraoral and oral ranges of

Table 13 Comparisons of extraoral and oral taste preferences

		Extraoral taste preferences		Oral taste preferences	
Amino acid (L-isomers)	Concentration (M)	Larvae	Juveniles	Larvae	Juveniles
Cysteine	0.1	20***	35***	7	80***
Histidine	0.1	20***	31***	9	28
Glutamine	0.1	17***	25***	7	36
Asparagine	0.1	14***	28***	4	35
Lysine	0.1	13***	29***	2	39
Glycine	0.1	13***	14	10	34
Methionine	0.1	11***	21**	19*	25
Serine	0.1	10***	24***	7	42
Threonine	0.1	9**	30***	10	43*
Phenylalanine	0.1	6	23***	4	41
Alanine	0.1	6	22***	7	16
Norvaline	0.1	6	7	5	15
Arginine	0.1	6	29***	6	42*
Proline	0.1	6	7	5	19
Valine	0.1	6	14	2	34
Glutamic acid	0.01	14***	36***	6	50*
Aspartic acid	0.01	11***	38***	7	69***
Isoleucine	0.01	6	15*	5	20
Tryptophan	0.01	6	23***	3	53**
Leucine	0.01	4	16*	4	26
Tyrosine	0.001	4	14	6	21
Control	–	5	10	7	23

Results from experiments on larvae (21–25 mm in body length) and juveniles (60–70 mm in body length) of Russian sturgeon, *Acipenser gueldenstaedtii* (modified from Kasumyan *et al.* 1992). Extraoral taste preferences are given as the mean number of snaps of agar pellets containing an amino acid. Oral taste preference is the mean percentage of pellets that were consumed in relation to number of snaps. 50 pellets were offered for 20 larvae or 10 juveniles. After 5 min remained pellets were counted and the percentage of consumed pellets per number of snaps was calculated. Pellets containing only dye (Cr_2O_3, 0.3%) were used as control, see text.

amino acids in sturgeon juveniles ($r_s = 0.79$; $P < 0.001$), while in sturgeon larvae, the extraoral and oral range of amino acids was not correlated ($r_s = 0.36$; $P > 0.05$). The extraoral range of free amino acids was similar in larvae and juveniles ($r_s = 0.67$; $P < 0.01$; Fig. 8). Morphological investigations show that the taste buds appeared at an earlier stage of sturgeon development in the barbels and lips than in the oral cavity (Devitsina and Kazhlaev 1995).

The results of behavioural assay have shown that fish larvae at the 'start feed' stage have the ability to discriminate among taste substances and release an adequate behavioural taste response. The early development of taste perception has been shown in many mammals (Formaker and Hill 1990) and in humans (Beauchamp *et al.* 1986, 1994). It has been suggested that the development of gustatory sensitivity to various substances may be conditioned by specific features of feeding and metabolism (Mistretta 1991). Nevertheless, taste perception in fish larvae is less well developed than in adult fish. The limited taste perception may be one reason for mass mortality of fish larvae and early postlarvae among capelin and Atlantic herring, which is caused by a dinoflagellate that is toxic to the fish (Gosselin *et al.* 1989). The toxin (saxitoxin) content of dinoflagellate reaches more than 10^{-4} μg cell^{-1} (Cembella *et al.* 1988). That amount of saxitoxin is enough to kill a first-feeding fish larva. The older fish are highly sensitive towards the toxins, which enable them to avoid poisonous food (Yamamori *et al.* 1988).

Ecology of taste preferences

In spite of the intensive investigations of taste perception in different groups of animals, mainly mammals, there is no clear concept explaining an animal's taste preference to some substances and the rejection of others. It has been suggested (Kassil 1972) that the

© 2003 Blackwell Publishing Ltd,

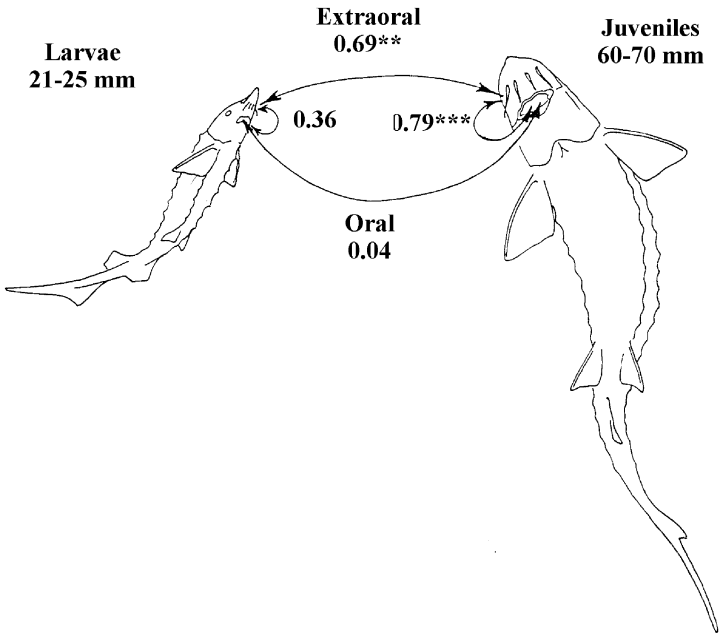

Figure 8 Oral and extraoral taste preferences. Values of Spearman's rank correlation coefficient between oral and extraoral taste preferences for free amino acids in larvae and juveniles of Russian sturgeon, *Acipenser gueldenstaedtii* (modified from Kasumyan 1999a).

palatability of substances (for mammals) might be related to the role these substances play in the metabolic processes. Nevertheless, such relationships seem to be very indirect and unpredictable.

Another explanation for taste preferences is based on the animal feeding ecology. According to general rules formulated with reference to numerous observations of the diets of various animals, herbivores manifested a preference for sugars more often than did nonherbivores (Harborne 1993). This rule seems to be applicable to fish also. It was shown that sucrose is highly palatable for grass carp, a herbivore fish, and for chub and roach (Kasumyan and Morsy 1997; Kasumyan and Nikolaeva 2002). Macrophytes, which are the main food for grass carp (Fischer 1973; Fowler *et al.* 1978) and filamentous algae are the preferable food for chub and roach during summer time (Boikova 1986; Specziar *et al.* 1997; Horppila *et al.* 2000). Sucrose is a stimulant for guppy (Kasumyan and Nikolaeva 1997), platy and black molly (an omnivorous poecilid fish that feeds on various aquatic invertebrates and plants; Dussault and Kramer 1981; Bailey and Sandford 1999). When cultivating guppy, it is recommended to include carbohydrate components like algae, leaves of lettuce or spinach and vegetative flakes into its diet to secure growth and reproduction (Pausan 1984; Stuart 1996).

Not all herbivorous fish have a preference for sucrose and other sugars. For example, the fraction of Romaine lettuce that contained sugars did not have any effects on the consumption of flavoured agar disks by herbivorous tilapia (Johnsen and Adams 1986). Moreover, some of the omnivorous and carnivorous fish have positive taste response for sucrose and other sugars. Such a preference was shown for shore rockling (Andriashev 1944), rainbow trout (Jones 1990), European eel, pike, zander, vendace (Appelbaum 1980) and brook char (Kasumyan and Sidorov 1998). However, for many omnivorous and carnivorous fish, sucrose was an indifferent taste substance. None of the fish species tested had been found to perceive sucrose as a deterrent (Table 4).

Based on a comparison of taste preferences in herbivorous and carnivorous fish, it was suggested that these two groups of fish have specific amino acids that stimulate feeding (Johnsen and Adams 1986). The authors claimed that amino acids found to be stimulants for carnivores failed to elicit feeding responses in the herbivores. However, this suggestion does not seem to be correct as grass carp, a herbivore fish, shows deterrent responses for substances such as L-glutamic acid, L-lysine, L-alanine, L-serine, which were highly palatable for tilapia (Johnsen and Adams 1986). L-Cysteine and glycine, the indifferent taste substances for tilapia, were stimulants for grass carp (Kasumyan and Morsy 1997; Kasumyan and Morsy 1998b). Many carnivorous fish show taste preferences for the same amino acids as do the herbivorous tilapia or grass carp (Table 7).

Many fish such as chub, brown trout and lake char are insectivores during late spring and summer time. For these fish, calcium chloride is a stimulant, but is a well-known bitter compound for humans (Kasumyan and Sidorov 1993a; Kasumyan 1997; Table 4). The taste preference for calcium chloride may be related to the important role that flying insects have as a food for these species. However, there are fish such as chum salmon and frolich char that also take flying insects as food and at the same time, do not show a taste preference for calcium chloride (Kasumyan and Sidorov 1993b, 1995d; Kasumyan 1997). Furthermore, the common carp and tench prefer the taste of calcium chloride, but do not feed on flying insects. Insects contain substances considered bitter by humans, but are readily eaten by insectivorous birds (Harborne 1993). The natural stimulants and deterrents in insects are not known. Both for the insectivorous birds and fish, the chemical nature of stimulants or deterrents is unknown.

Internal factors effecting taste preferences

Feeding experience

It is well known that fish release strong and obvious behavioural responses to feeding signals emanating from common food objects. This has been shown for food stimuli mediated by olfaction and vision (McBride *et al.* 1962; Atema *et al.* 1980; Colgan *et al.* 1986; Kasumyan and Ponomarev 1986; Stradmeyer and Thorpe 1987; Meyer 1988). However, when it comes to taste preferences, the dependence upon feeding experience has been shown to be less prominent (Kasumyan and Morsy 1997, 1998b). In these studies, grass carp siblings were used. Grass carp larvae at the 'start feed' stage were obtained from a fish farm (Krasnodar district) and were raised on planktonic *Cladocera* and the artificial feed 'TetraMin' (Tetra Co., Germany) up to 3 months of age at which the fish had attained a body length of 2.5 cm. Then, the fish were divided into two groups, each group being fed a distinct diet for 6 months. A 'carnivorous' group of grass carp siblings was reared on live mosquito larvae, while a 'vegetarian' group was reared on duckweed and Romaine lettuce leaves. Both types of conditioning diets were adequate for grass carp juveniles because in nature, zoophagy is combined with macrophytophagy (Fischer 1973; Fowler *et al.* 1978). At the end of the rearing period, the 9-month-old grass carps in both the groups were 6–9 cm in body length.

It was found that after 6 months, the grass carp juveniles of the 'herbivorous' and the 'carnivorous' groups had similar taste preferences for the four classical taste substances and the 21 amino acids tested (Fig. 9). Pellets containing sucrose, cysteine, citric acid, aspartic acid or glycine were highly palatable substances. Seventeen of the 21 free amino acids tested were deterrent substances for grass carp siblings of both the groups. A highly significant positive correlation was found for taste preferences between the herbivorous and carnivorous groups of fish to the taste substances tested ($r_s = 0.74$; $P < 0.001$).

However, the taste preferences of the fish were not identical, and differences in taste preferences between the two groups were found. Pellets containing calcium chloride or sodium chloride were consumed more readily in fish of the 'herbivorous' group than in fish of the 'carnivorous' group. The difference between the acceptance ratios of control and flavoured pellets was statistically significant only for the 'herbivorous' group. The differences in taste preferences of calcium chloride and sodium chloride between 'carnivorous' and 'herbivorous' fish were not significant. In each group of grass carp, pellets containing the water extract of conditioning diet were preferred to those containing the extract of unconditioning diet (both 75 g L^{-1}). Pellets with water extracted from mosquito larvae were consumed 1.27× more readily than pellets flavoured with water extracted from Romaine lettuce leaves in 'carnivorous' fish and 1.22× less readily in 'herbivorous' fish. The consumption of pellets flavoured with mosquito larvae or plant extracts differed significantly between these two groups ($P < 0.001$ and $P < 0.01$, respectively).

These observations suggest that conditioning with a long-term rearing on a selected food leads to only a small shift in taste preferences and only a slight increase in palatability for the substances in the food on which they were reared. In conclusion, the results from these experiments provide evidence that taste preferences show low plasticity in fish. In other words, taste preference does not depend upon the fish diet. The fish diet might be reflected in taste preferences of fish to food extracts, which have a more complicated composition. The results show that taste preference in fish is under strict genetic control. Based on these results and the previously discussed data, it seems reasonable to conclude that taste preference in fish is a characteristic feature of the species and is independent of population, sex and previous feeding experience.

© 2003 Blackwell Publishing Ltd,

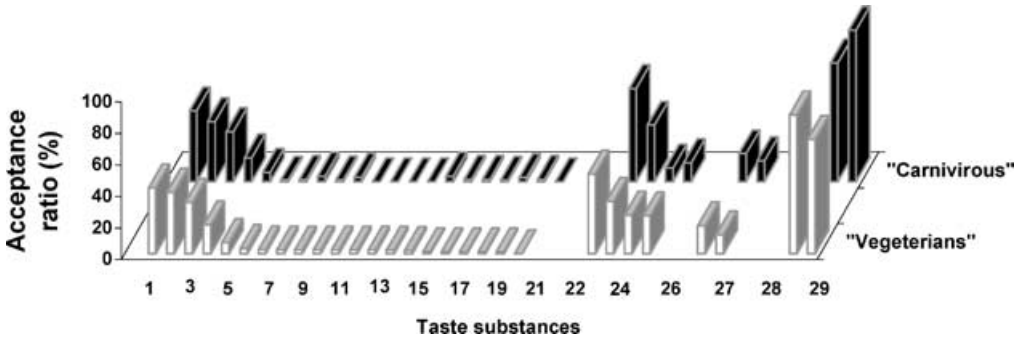

Figure 9 Effect of feeding experience on taste preferences. Taste preferences to free amino acids, classical taste substances and food extracts in grass carp (*Ctenopharyngodon idella*) juveniles reared on different diets. 1 – L-Alanine, 0.1 M; 2 – L-arginine, 0.1 M; 3 – L-asparagine, 0.1 M; 4 – L-aspartic acid, 0.01; 5 – L-valine, 0.1 M; 6 – L-histidine, 0.1 M; 7 – glycine, 0.1 M; 8 – L-glutamine, 0.1 M; 9 – L-glutamic acid, 0.01 M; 10 – L-isoleucine, 0.01 M; 11 – L-leucine, 0.1 M; 12 – L-lysine, 0.01 M; 13 – L-methionine, 0.1 M; 14 – L-norvaline, 0.1 M; 15 – L-proline, 0.1 M; 16 – L-serine, 0.1 M; 17 – L-tyrosine, 0.001 M; 18 – L-threonine, 0.1 M; 19 – L-tryptophan, 0.01 M; 20 – L-phenylalanine, 0.1 M; 21 – L-cysteine, 0.1 M; 22 – sucrose, 0.29 M; 23 – citric acid, 0.26 M; 24 – calcium chloride, 1.73 M; 25 – sodium chloride, 0.9 M; 28 – water extract of Romaine lettuce (*Lactuca sativa*) leaves, 75 g (wet weight) L^{-1}; 29 – *Chironomidae* larvae water extract, 75 g (wet weight) L^{-1}; 26 and 27 – controls (blank pellets) for 'free amino acid' series and for 'classical taste substances and food extracts' series, respectively (modified from Kasumyan and Morsy 1997b).

Feeding motivation

The fish feeding motivation strongly influences taste preferences. It was found that starvation during 17–18 hours caused a widening of the extraoral taste spectrum in 4–6 cm long Siberian sturgeon juveniles in comparison with fish which had food *ad libitum* 1.0–1.5 hours before behavioural trials. The number of substances effective for oral taste reception changed relatively little (Table 14). The water temperature was 18 °C during the experiments (Kasumyan 1997).

Environmental factors affecting taste preferences

Temperature

The ambient water temperature is one of the most potent abiotic variables affecting vital functions in exothermic animals like fish. Temperature also has an effect on taste preferences in fish. The grass carp shows different levels of relative food preference for the same objects in warm (20 °C) and cold (13 °C) water (Adamek *et al.* 1990). A study on the effect of water temperature on fish taste responses was performed on juvenile stellate sturgeon 4–5 months in age and 6–8 cm in body length (Kasumyan *et al.* 1992). Two groups of fish were held separately in different water temperatures for 5 days before starting the bioassays. In the 'warm' series, the water temperature was 20 °C and in the 'cold' series, it was 12.5 °C. In the 'warm' series, 19 of 21 free amino acids tested were incitants for the extraoral gustatory system of stellate sturgeon, and caused significant increase in the number of snaps at agar pellets (Table 15). The three amino acids, L-alanine, L-histidine and L-cysteine, were stimulants and caused a considerable increase in the number of pellets consumed in relation to the number of pellets snapped. A significant correlation was found between extraoral and oral taste responses ($r = 0.49$; $P < 0.05$). In the 'cold' series, 17 of 21 free amino acids were incitants for fish, and two amino acids, L-histidine and L-valine, were stimulants. No significant correlation was found between extraoral and oral taste responses of fish in the 'cold' series ($r = 0.20$; $P > 0.05$). No significant relationship between oral taste responses of fish in the 'warm' and the 'cold' series was found ($r = 0.20$; $P > 0.05$), although extraoral taste responses obtained at different water temperatures were highly correlated ($r = 0.92$; $P < 0.001$). The mean number of pellets snapped and swallowed was significantly higher in the 'warm' series. These results confirm those obtained by other authors concerning the effect of water temperature on fish feeding activity (Knights 1985; Metcalfe *et al.* 1986; Stradmeyer and Thorpe 1987).

The difference in water temperature between the 'warm' and 'cold' series (7.5 °C) is enough to change the behavioural responses to chemical signals in ectothermic animals (Yamashita 1964; Flerova and Gdovskii 1976; Jones 1980; Jones 1983; Van Damme *et al.* 1990). The extraoral taste responses in the

Table 14 The effect of starving on extraoral and oral taste preferences

		Extraoral taste preferences, food deprivation for		Oral taste preferences, food deprivation for	
Amino acid (L-isomers)	Concentration (M)	1–1.5 hours	17–18 hours	1–1.5 hours	17–18 hours
Threonine	0.1	23*	42***	60	87***
Asparagine	0.1	21	47***	69**	62*
Glycine	0.1	16	36***	63*	71***
Methionine	0.1	14	41***	49	56
Glutamic acid	0.01	9	32***	46	70**
Control	–	10	15	52	49

Juveniles of Siberian sturgeon, *Acipenser baerii* (4–6 cm in body length) were tested after being starved for one to one and a half hour or 17–18 hours. Extraoral taste preference is the mean number of snaps of agar pellets containing free amino acids. Oral taste preference is the mean percentage of pellets that were consumed in relation to number of snaps. 50 pellets were offered to 5 juveniles. After 5 min remained pellets were counted and the percentage of consumed pellets per number of snaps was calculated. Pellets containing Cr_2O_3 were used as Controls. Note the increase in snaps and consumption with duration of starvation time.

Table 15 Palatability and water temperature

		Extraoral taste preferences		Oral taste preferences	
Amino acid (L-isomers)	Concentration (M)	12.5 °C	20 °C	12.5 °C	20 °C
Cysteine	0.1	32	35	15	60***
Lysine	0.1	29	28	11	30*
Serine	0.1	25	28	8	24*
Threonine	0.1	22	22	5	17
Histidine	0.1	21	27*	30	57*
Asparagine	0.1	20	21	15	21
Arginine	0.1	19	25	9	11
Methionine	0.1	19	20	3	15
Glutamine	0.1	16	23**	7	12
Phenylalanine	0.1	15	18	11	16
Alanine	0.1	14	24**	9	60***
Valine	0.1	10	12*	27	12
Glycine	0.1	10	19**	14	33**
Norvaline	0.1	8	11*	9	15
Proline	0.1	3	10***	5	15
Aspartic acid	0.01	33	36	11	24
Glutamic acid	0.01	18	28**	18	7*
Isoleucine	0.01	10	14	15	16
Leucine	0.01	10	12	17	15
Tryptophan	0.01	5	13***	18	15
Tyrosine	0.001	3	9***	0	14
Control	–	6	7	6	16

Effect of water temperature on extraoral and oral taste preferences for free amino acids in stellate sturgeon, *Acipenser stellatus*. Extraoral taste preference is the mean number of snaps of pellets containing free amino acids. Oral taste preference is the mean percentage of pellets that were consumed in relation to number of snaps. Fifty pellets were offered for 5 fish. After 5 min remained pellets were counted and the percentage of consumed pellets per number of snaps was calculated. Pellets controls contained 0.5 mM of the red colour Ponceau 4R (modified from Kasumyan *et al*. 1993).

© 2003 Blackwell Publishing Ltd,

stellate sturgeon did not undergo any appreciable modification at different temperatures, while the oral taste preferences did. Of particular interest is the fact that the rank order of stimulatory efficiency differed between the amino acids under different temperature conditions. Amino acids like histidine, asparagine, phenylalanine, isoleucine, norvaline and proline kept their rank order at the two temperatures, whereas glutamic acid, alanine, tryptophan, valine and leucine changed their rank drastically (Table 15). Temperature dependence of taste responses to taste substances of the same type is expressed differently in different animal species (Yamashita 1964; Nakamura and Kurihara 1991). We suggest that temperature-dependent taste responses might be important for fish and facilitate their seasonal shifts in feeding regime.

Water pollutants

Fish taste receptors are exposed to the environment and predisposed to the detrimental effects of water pollutants. It has been shown that many pollutants, especially heavy metals, affect fish taste reception by both destroying the taste buds and reducing the sensitivity to the taste stimuli. These events occur after a short exposure of the fish to polluted water (for review, see Brown *et al.* 1982; Klaprat *et al.* 1992).

There have been few experiments on the effect of heavy metals on the behavioural taste response in fish (Kasumyan 1997; Kasumyan and Morsy 1998a). The effects of different heavy metals on taste behavioural responses of 9–12-cm long common carp, after 1-, 3-, 6-, 24-, 48- and 72-hour exposures in solutions of $HgCl_2$, $CuSO_4$, $CdSO_4$, $ZnSO_4$ and $Pb(NO_3)_2$ were investigated. The amino acids L-cysteine (0.1 M), L-glutamic acid (0.01 M) and water extract of chironomid larvae (75 g L^{-1}) were used as taste stimuli. It was found that in clean water, the unexposed fish swallowed 99 and 52% of agar–agar pellets containing either L-cysteine or L-glutamic acid. The negative effect of heavy metals was evident after 15-min exposure. The exposure of fish during 1–3 hours in heavy metal salt solution (1 μM) provides a strong and deep suppression on the taste responses of fish. Under these conditions, the fish more often rejected the pellets containing amino acids with very high palatability than did individuals in a control situation. The exposed fish accepted few pellets, and the retention time of pellets decreased, althougth feeding motivation did not change, and all pellets offered were still actively grasped by fish after 3, 6 or 24 hours of exposure. The different heavy metals had different suppression intensities on taste responses at 1 μM. The strongest effect was induced by $HgCl_2$; $CuSO_4$ was somewhat less effective than $HgCl_2$, and the weakest effects were produced by $CdSO_4$, $Pb(NO_3)_2$ and $ZnSO_4$ (Fig. 10). As the $HgCl_2$ concentration decreased from 1 μM to 1 nM, the time to observe a change in taste responses increased from less than 1 hour to 24 or 72 hours, depending on the palatability of the amino acids used (Fig. 11).

The loss of taste responses in fish after the exposure to heavy metals is reversible. Some time after the cessation of exposure to toxic substances, the taste responses of fish improve to their former function. In common carp, the taste responses after an exposure to 0.1 μM $HgCl_2$ for 3 hours were recovered after 6–12 days at a temperature of 20 °C (Fig. 12), and there appeared to be a direct relationship between the duration of the exposure to a toxic substance and the duration of the recovery period.

Low pH water

European grayling juveniles, 6–8 cm in length, were used as subjects for studying the effect of low pH in the water on taste preferences in fish. Taste preferences were estimated for L-isomer amino acids, and classical taste substances and the pH of the tank water were lowered by the addition of sulphuric acid.

In natural waters, with a pH between 7.6 and 7.8, juvenile grayling demonstrated significant taste preferences to pellets containing all the classical taste substances used. In behaviour assays, it was shown that most of the 21 L-isomers of amino acids in the concentration range of 10^{-1} to 10^{-3} M were either ineffective or were deterrent stimuli to the grayling. It was shown that only five amino acids increased the consumption of pellets by grayling. When grayling were exposed to water with a pH of 6.0, the pellet consumption decreased in the first 3–6 hours. The pellet consumption increased slowly up to 24–48 hours, but decreased again after 72-hour exposure. When the fish were exposed to a pH of 5.3, the pellet consumption decreased to about half of the normal consumption after 3 hours, and to 20% after 72 hours. The fish mortality was not evident in water at pH 6.0 and 5.3 and with a 72-hour exposure. These results show that short-time exposure in water with a low pH can induce drastic changes in the ability of fish to respond to taste substances (Kasumyan and Sidorov 1995b). This finding might explain the misbalance in biotic communities of acidified water.

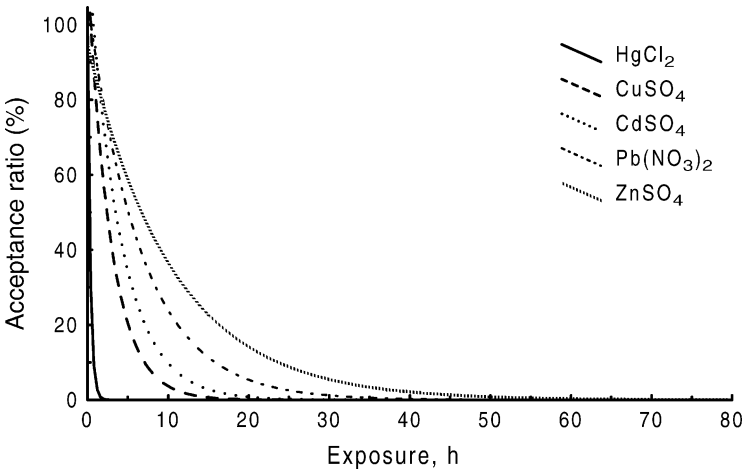

Figure 10 Effect of heavy metals on taste preferences. Taste preferences to L-cysteine (0.1 M) in common carp (*Cyprinus carpio*) exposed to solutions of five different heavy metals at 1 μM (modified from Kasumyan and Morsy 1998a).

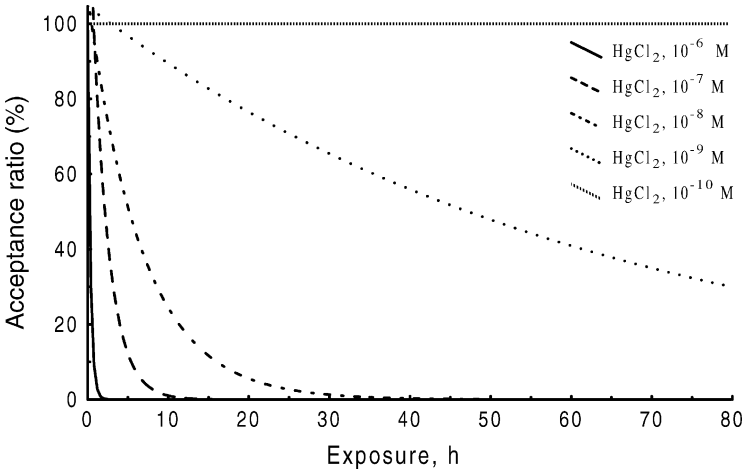

Figure 11 Effect of mercury on taste preferences. Taste preferences to L-cysteine (0.1 M) in common carp (*Cyprinus carpio*) exposed to mercury chloride solutions at five different concentrations (modified from Kasumyan 1997).

Taste preferences as a function of pH

No clear relationship was found between the pH of a test solution and its palatability, when using amino acids as taste substances (Jones 1989). Eighty-one solutions of various amino acids (ranged from pH 3.06, by adding 0.01 M L-aspartic acid, to pH 10.87, gained from the water containing 1.0 M L-arginine) were tested on rainbow trout. The most effective solutions lay predominantly in the range of pH 5.8–6.6, but this predominance was probably weighted by virtue of the sheer number of solutions

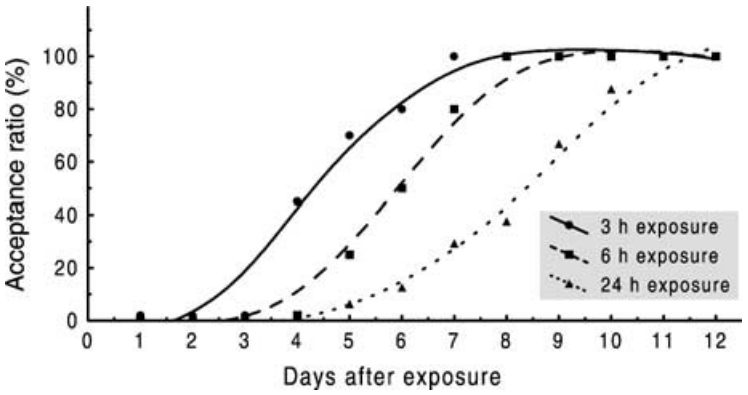

Figure 12 Recovery of taste preferences. Recovery of taste preferences to L-cysteine (0.1 M) in common carp (*Cyprinus carpio*) after exposure to mercury chloride solution (0.1 μM; modified from Kasumyan 1997).

© 2003 Blackwell Publishing Ltd,

with pH in this range. Aqueous solutions of those compounds which were palatable, as well as those which were not, ranged in pH from quite acidic to quite basic. For instance, the acidic glutamic acid, the near-neutral proline and the basic arginine were clearly active. In contrast, neither the more acidic L-aspartic acid, the near-neutral glycine and L-alanine, nor the basic L-histidine showed any activity. Based on these results, it was concluded that there is no direct relationship between pH and palatability, nor were there distinct pH thresholds above or below which the amino acids became active. Instead, as a class, the amino acids displayed activity over a wide range of pH. This is not to say that the activity of specific amino acids is always pH-independent. The possibility remains that the pH dampens and/or enhances the effects of particular amino acids by influencing their binding kinetics with available gustatory cell receptor sites, and thereby changes their perceived intensity and/or quality. Electrophysiological recordings do, however, indicate that at least the magnitude of the gustatory response to amino acids is relatively pH-independent. Except for arginine, the response of the rainbow trout palatine to highly stimulatory amino acids is constant at pH above 7 (Marui *et al.* 1983a). In common carp, taste response magnitudes are constant above pH 6.0 (Marui *et al.* 1983b), and the Japanese eel palatine is equally sensitive to glycine at pH 5 and 9 (Yoshii *et al.* 1979). At pH below 7, the gustatory response magnitudes of both rainbow trout and common carp increase dramatically, but these changes were in parallel to the increased response to acidified natural water alone (Marui *et al.* 1983a,b). The latter response is probably because of an elevated CO_2 tension. The taste system of fish is highly sensitive to CO_2 (Konishi *et al.* 1969; Yoshii *et al.* 1980). No differences were found in rainbow trout tested electrophysiologically by recording from the palatine nerve and stimulating with free amino acids at two different pH ranges, the local natural pH of 6.6–6.8 and 7.3–7.8, which were achieved by using a limestone pellet filter (Hara *et al.* 1999).

To measure the effect of pH on the feeding stimulation of fish, a series of substances such as carboxylic acids and amino acids (0.01 M) at the same concentration but with different pH were tested on tilapia. The relationship of pH to palatability was nonlinear. Moreover, the results indicate an optimum pH, where the palatability of food was the highest and which decreased if the pH was shifted from this value. It was suggested that acidity might be one but not the sole controlling factor of food palatability for this fish (Adams *et al.* 1988). The experiments on juveniles of European grayling revealed a weak but significant negative relationship between pH of solutions (0.1 M) and the palatability of 14 carboxylic acids ($r_s = -0.69$; $P < 0.01$; Kasumyan 1997). The palatability for 19 organic acids, mainly carboxylic acids, was determined for three cyprinids: tench, bitterling and dace. The Spearman's rank correlation coefficient between the acceptance ratio of pellets and the pH of the solution of taste substances was highly positive for bitterling ($r_s = 0.91$; $P < 0.01$), slightly positive for dace ($r_s = 0.48$; $P < 0.05$) and highly negative for tench ($r_s = -0.88$; $P < 0.01$; Fig. 13; Kasumyan and Prokopova 2001; Kasumyan and Fokina, unpublished). Thus, it seems obvious that the relationship between palatability and pH is different in different fish species.

Chemical nature of stimulants in fish food organisms

There are two approaches in studying the chemical nature of the taste stimulants. The first consists of screening of substances that are isolated and identified in various fish food organisms by analysing their chemical composition (Konosu *et al.* 1965; Carr *et al.* 1977; Miyagawa *et al.* 1979; Mackie and Mitchell 1983; Iida *et al.* 1992; for review, see Carr *et al.* 1996). This approach is the most common in studying fish taste preferences and in the search for fish taste stimulants (Mackie 1982; Ikeda *et al.* 1988a,b; Takaoka *et al.* 1990).

Using this approach, it was shown that many amino acids are highly palatable for fish and are the main components responsible for the palatability of fish food organisms. For example, a mixture containing all the amino acids found in the marine annelid had the same effect on feeding intake as a complete synthetic extract for both Japanese eel and red sea bream. The active constituents in the amino acids fraction was identified as glycine, L-alanine, L-proline and L-histidine for Japanese eel (Takeda *et al.* 1984), and glycine, L-alanine, L-lysine, L-valine, L-glutamic acid, and L-arginine for red sea bream (Fuke *et al.* 1981). A mixture of L-amino acids from the synthetic squid extract was stimulatory for European eel and rainbow trout, while the corresponding D-amino acids were ineffective taste substances (Mackie and Mitchell 1983) or deterrents (Adron and Mackie 1978) for these fish. For rainbow trout, the aromatic and basic amino acids of a squid extract acted as a feeding

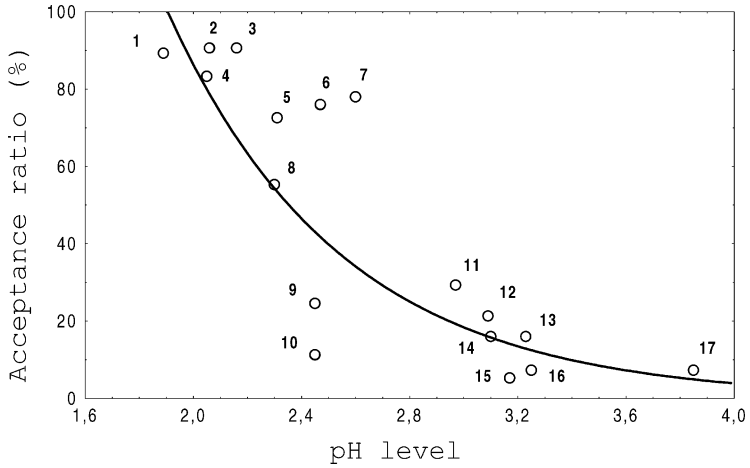

Figure 13 Taste preference and pH. Relationship between pH and taste preferences for organic acids at a concentration of 0.1 M in tench (*Tinca tinca*). 1 – Oxalic acid; 2 – maleic acid; 3 – α-ketoglutaric acid; 4 – tartaric acid; 5 – malonic acid; 6 – citric acid; 7 – malic acid; 8 – glycolic acid; 9 – fumaric acid; 10 – ascorbic acid; 11 – succinic acid; 12 – adipic acid; 13 – butyric acid; 14 – valeric acid; 15 – acetic acid; 16 – propionic acid; 17 – formic acid (from Kasumyan and Prokopova 2001).

stimulant, while fractions containing neutral and acidic amino acids were inactive. The most efficient stimulants among the amino acids were L-tyrosine, L-phenylalanine, L-lysine and L-histidine (Adron and Mackie 1978). High palatability of free amino acids was found for various freshwater and marine fish by many researchers (Hidaka 1982; Mackie 1982; Mearns *et al.* 1987; Adams *et al.* 1988; Jones 1989; Kasumyan 1997).

The free amino acids are not, however, solely responsible for the high palatability of food organisms for fish. For both plaice and dab, the L-amino acid components and the nonamino acid components of synthetic squid mixture acted as feeding stimulants (Mackie 1982). For Atlantic cod, a mixture of both L-amino acids and neutral L-amino acids acted as stimulants, but neither were as effective as the synthetic squid mixture. A diet containing 1% of a synthetic squid extract was consumed to 100%, nonamino acid fraction was not consumed, all the amino acids were consumed at 61%, and the neutral amino acids were consumed at 48% (Johnstone and Mackie 1990).

The second approach to the study of the chemical nature of taste stimulants consists of separation of extract of food organisms and analysing the composition of chemical fractions that show stimulatory effect in the bioassay. It should be noted that this approach is very promising for studies on the chemical nature of taste stimulants, but has seldom been used (Johnsen and Adams 1986; Takii *et al.* 1986; Mearns *et al.* 1987).

The assessment of palatability for agar gels flavoured by chemical fractions of aqueous extract of shrimp was made for two salmonid fish, Atlantic salmon and rainbow trout (Mearns *et al.* 1987). The results indicate that various types of substances are stimulants for rainbow trout. These are free amino acids, nucleotides, ammonium bases and some acidic and neutral compounds, which were not identified. By contrast, the Atlantic salmon appears to be a selective feeder. Agar gel pellets flavoured by the stock solution containing all water-extractable compounds, but without protein, were only accepted to a certain degree, and a high proportion of pellets were rejected. It was supposed that the water-soluble proteins, which were removed in the first stage of the fractionation procedure, are responsible for the palatability of shrimp extract. Further studies are required to investigate this possibility.

In an attempt to find stimulants for herbivorous tilapia, the aqueous extract of Romaine lettuce was fractionated and a bioassay of chemical fraction was performed. Contrary to Atlantic salmon, agar gel flavoured with extract depleted of proteins by precipitation with TCA was consumed to the same degree as agar gel flavoured with whole lettuce homogenate, indicating that proteins are not the stimulants. The lipid-containing ether extract as well as the fractions containing organic acids or sugars also had no stimulant activity. The free amino acid fractions only elicited positive taste response in tilapia. The five amino acids – glutamic acid, aspartic acid, serine, lysine and alanine – were found to be stimulants for

 © 2003 Blackwell Publishing Ltd,

tilapia (Johnsen and Adams 1986). Further studies showed that citric acid was one of the major constituents of the organic acid fraction, and was found to be highly palatable for tilapia when examined as single taste substances (Adams *et al.* 1988).

Various separation methods were used for identification of feeding stimulants for young yellowtail to jack mackerel muscle extract (Hidaka *et al.* 2000). The authors found that the stimulatory substances had a molecular weight less then 10 000 and that the substances were absorbed to the stationary phase in an anion-exchange column (DEAE-Sephadex). Stepwise elution with NaCl and testing of fractions in starch pellets indicated that inosine-5′-monophosphate and lactic acid are the main feeding stimulatory substances for yellowtail.

Relation between oral and extraoral taste preferences

There are only a few studies that permit comparisons of the oral and extraoral taste systems. They have all been done on sturgeons. Sturgeons have numerous taste buds, not only in the mouth, but also on the epidermis of both barbels and lips (Pevzner 1981; Devitsina and Kazhlaev 1993). As a result of the external gustatory receptors on the barbels, sturgeon can receive information concerning palatability of food items without taking them into the mouth. A rapid touch of a food item by one barbel is sufficient to assess the food quality. This preliminary assessment of food, which takes place in a fraction of a second, seems to be essential for inducing feeding behaviour in sturgeon. The final assessment of food acceptability is based on the oral sense (Kasumyan and Kazhlaev 1993a; Kasumyan 1999a).

In 4–6-cm long Siberian sturgeon, 14 of 19 amino acids were incitants, in 6–7-cm long Russian sturgeon, 16 of 21 were incitants and in 6–8-cm long stellate sturgeon, 19 of 21 amino acids gave a significant increase in the number of food pellets caught (Table 16). Twelve of these amino acids were common to the three sturgeon species, although the correlation coefficient was high only for Russian sturgeon versus stellate sturgeon (the Spearman's rank correlation coefficient $r_s = 0.84$; $P < 0.001$). The most potent incitants for these two species were L-aspartic acid and L-glutamic acid and L-cysteine, while L-asparagine, L-threonine and L-methionine were the most potent substances for Siberian sturgeon. Only two amino acids, namely L-proline and L-tyrosine, were ineffective for all three species.

The oral taste system seems to have a narrower spectrum of effective amino acids than the extraoral taste system. There were only six substances found to be stimulants for the Russian sturgeon, three for the stellate sturgeon and seven for the Siberian sturgeon. Alanine had a deterrent effect in this last species (Table 7). Among the stimulant amino acids, none were common to all the three species, and there was no significant correlation between the species towards responses to amino acids. There was no significant correlation between sturgeon species concerning the oral taste responses. Comparison of the extraoral and oral taste responses within one species revealed a high correlation in the Russian sturgeon ($r_s = 0.79$; $P < 0.001$) and lower correlation in the Siberian sturgeon ($r_s = 0.48$; $P < 0.05$) and stellate sturgeon ($r_s = 0.44$; $P < 0.05$). It is noteworthy that some substances have an opposite effect on extraoral and oral taste receptors. For example, citric acid is a strong incitant for sturgeons and increases the frequency of pellets caught by fish in comparison with blank pellets. But all pellets with citric acid are immediately rejected.

The extraoral taste system is at least 10 times more sensitive than the oral system. For example, the extraoral taste threshold for citric acid is 5 mM and the oral taste threshold is 50 mM (Fig. 14; Kasumyan and Kazhlaev 1993a). Calculations based on the size of pellets revealed that about 1–2 μg per pellet of taste substance is enough to stimulate appetite in fish.

The extraoral taste system develops more rapidly than the oral system during sturgeon ontogenesis. Eleven amino acids were effective for extraoral taste buds in Russian sturgeon larvae (2.1–2.5 cm in body length) compared with 16 amino acids in juveniles (6–7 cm in body length). In the oral taste system, the difference between larvae and juveniles was much more dramatic, with only one amino acid being effective for larvae compared with six in juveniles (Table 13). There was no correlation in the oral taste spectra of larvae and juveniles, whereas the correspondence in extraoral taste spectra of larvae and juveniles was very high ($r_s = 0.69$; $P < 0.01$; Kasumyan *et al.* 1992).

According to the phylogenetic tree of sturgeons drawn up on the basis of their karyotypes, the Russian sturgeon and Siberian sturgeon with 240–260 chromosomes evolved more than 80 million years ago (Vasil'ev 1985). The stellate sturgeon, with 120 chromosomes, evolved significantly earlier. Based on these aspects of sturgeon phylogeny, it was

Table 16 Extraoral taste preferences to amino acids

Amino acid (L-isomers)	Concentration (M)	Russian sturgeon	Stellate sturgeon	Siberian sturgeon
Cysteine	0.1	35**	35***	–
Histidine	0.1	31***	27***	24*
Threonine	0.1	30***	22***	42***
Arginine	0.1	29***	25***	17
Lysine	0.1	29***	28***	22
Asparagine	0.1	28***	21***	47***
Glutamine	0.1	25***	23***	29***
Serine	0.1	24***	28***	28***
Phenylalanine	0.1	23**	19***	22*
Alanine	0.1	21**	24***	30***
Methionine	0.1	16*	20***	41***
Glycine	0.1	14	19***	36***
Valine	0.1	10	12**	30***
Norvaline	0.1	7	11**	–
Proline	0.1	7	10	15
Aspartic acid	0.01	38***	36***	26**
Glutamic acid	0.01	36***	28***	32***
Tryptophan	0.01	23***	13***	32***
Leucine	0.01	15*	12**	30***
Isoleucine	0.01	14	14***	14
Tyrosine	0.001	14	9	19
Control	–	7	7	15

Three species of *Acipenser* sturgeons: Russian sturgeon, *A. gueldenstaedtii* (6–7 cm body length), stellate sturgeon, *A. stellatus* (6–8 cm body length) and Siberian sturgeon, *A. baerii* (4–6 cm body length) were exposed to pellets containing amino acids. Extraoral taste preference is the mean number of snaps of a 50 agar pellets by five fish during 5-min exposure time. Controls were pellets containing Ponceau 4R or 0.3% Cr_2O_3 (modified from Kasumyan 1999a).

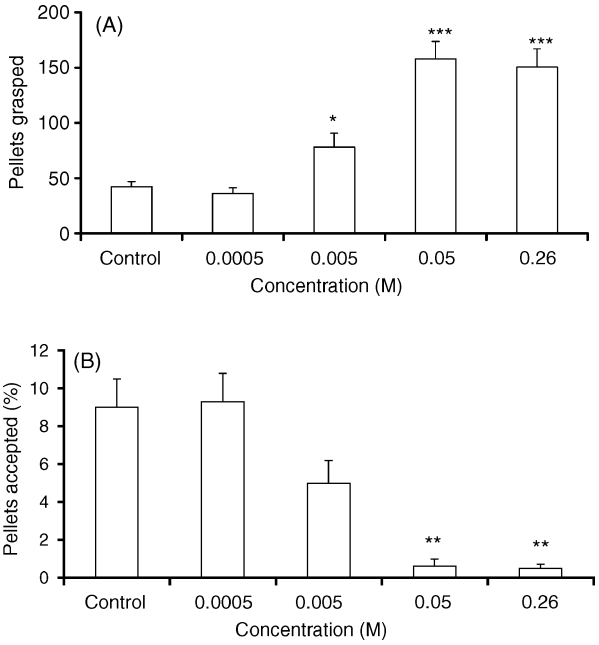

Figure 14 Oral and extraoral taste preferences. Oral (A) and extraoral (B) taste preferences for different concentration of citric acid in juveniles of Siberian sturgeon, *Acipenser baerii*. Control – blank pellets. Vertical columns and T-bars are the mean and the SEM (modified from Kasumyan and Kazhlaev 1993).

© 2003 Blackwell Publishing Ltd,

suggested that extraoral taste sense is evolutionarily more stable than oral taste sense (Kasumyan 1999a,b).

Correlation between bioassay and electrophysiological data

The input to the brain from any sensory structure can be recorded by electrophysiological means. These methods are powerful techniques as they can reveal thresholds or sensitivities, specificity, adaptation and many other functional properties of these systems. However, the recordings of nervous activity does not tell about the animals' reaction towards a specific chemical; in other words, a discharge of nerve impulses can indicate that the stimulus was either a stimulant or a deterrent. It seems important to relate the activity evoked by taste substances determined by electrophysiological methods and those determined in bioassays. Such comparisons, however, are available for a few species only.

In the red sea bream, a least square correlation coefficient of 0.78 was found between amino acids that were effective in initiating feeding behaviour and those that stimulated gustatory neural activity (Goh and Tamura 1980a). In the puffer, the substances L-alanine, glycine, L-proline, L-serine and betaine stimulated lip taste receptors in electrophysiological recordings. These substances were also effective in stimulating pellet acceptance. The concentration of amino acids needed to release behavioural responses was greater by two to three orders of magnitude than that required to elicit gustatory neural activity (Hidaka *et al.* 1978; Ohsugi *et al.* 1978). In both the electrophysiological studies and in the behavioural tests, the long-chained aliphatic organic acids – butyric, caproic and valeric acids – were found to be stimulatory substances for Atlantic salmon. Recordings from the palatine nerve showed that, of the few amino acids tested, all were nonstimulatory, with the exception of L-proline. However, L-proline was an indifferent taste substance for Atlantic salmon in bioassays (Sutterlin and Sutterlin 1970).

There are several examples of discrepancies between the sensory input revealed by electrophysiological means and the behavioural output evoked by the same substances. In Arctic char, L-glutamic acid was found to be the most palatable substance in behavioural studies (Kasumyan and Sidorov 1995d), but had no effect in electrophysiological recordings (Hara *et al.* 1993). In contrast, L-proline and L-alanine were the most effective taste stimuli in electrophysiological study for Arctic char (Hara *et al.* 1993), but were classed as neutral taste substances in behavioural assays (Kasumyan and Sidorov 1995d). Hydroxy-L-proline evoked intense neural responses in rainbow trout palate nerve (Marui *et al.* 1983a), but was not a palatable taste substance for this fish (Jones 1990). Similar observations were made for L-alanine and betaine, which were ineffective taste substances in behavioural assay (Jones 1989), but elicited strong neural responses in the rainbow trout palate (Marui *et al.* 1983a).

It has been shown that L-proline is the only amino acid that evoked responses from the gustatory nerves in electrophysiological studies in seven Salmonidae species and in nine strains of the rainbow trout (Hara *et al.* 1999). The detection threshold for L-proline was found to be between 10^{-7} and 10^{-8} M. These characteristic features are consistent with the hypothesis proposed by Hara and coworkers (Hara and Marui 1984; Hara *et al.* 1993) that 'Pro' receptor or transduction system is one of the four systems that mediate stimulation by amino acids in salmonids. However, proline was an ineffective stimulus in the behavioural assay provided on different salmonids – chum salmon (Kasumyan and Sidorov 1993b), brown trout (Kasumyan and Sidorov 1995c), frolich char (Kasumyan and Sidorov 1995d) – and proline was found to be a deterrent for rainbow trout by Adron and Mackie (1978), whereas it was found to be a highly palatable substance by Jones (1989). Nucleotides AMP, ADP, IMP and UMP have been shown to be effective taste stimuli in neural recordings in the puffer, but these compounds did not evoke positive taste responses in behavioural assays performed on that species (Hidaka *et al.* 1977; Hidaka 1982). Discrepancies between the sensory input revealed by electrophysiological means and the behavioural output emphasise that palatability is not synonymous with excitability in the fish taste system.

Correlation between smell and taste preferences

Olfaction and gustation are the main chemosensory systems in fish. Both these systems are sensitive to chemical substances released by fish food organisms and play important roles in fish feeding behaviour (Atema 1980; Døving 1986; Pavlov and Kasumyan 1990, 1998; Marui and Caprio 1992). There is a distinct difference in the anatomy of the gustatory and olfactory organs in fish, and these distinctions are also

evident when it comes to the functional characteristics of these chemosensory systems. According to electrophysiological studies, the gustatory system tends to be more selective than the olfactory system, and the gustatory spectra of effective taste substances are more species-specific than the olfactory spectra (Goh and Tamura 1980b; Døving 1986; Caprio 1988; Marui and Caprio 1992; Hara 1994a).

Bioassays support the notion that there are specialisations of the olfactory and gustatory systems in fish. The effectiveness of amino acid solutions in evoking feeding behaviour in common carp via the olfactory system (Saglio and Blanc 1983; Kruzhalov 1986) is different from the palatability of these substances (Kasumyan and Morsy 1996), and demonstrates that the responses of the gustatory and olfactory systems are quite different. The spectra of palatable amino acids (Kasumyan and Sidorov 1995c) do not coincide with the spectra of amino acids efficient as olfactory stimuli for brown trout (Mearns 1986). Glycine, which evoked obvious bottom food searching behaviour in different species of sturgeons (Acipenseridae) was an oral taste stimulant for the Siberian sturgeon only (Kasumyan 1994; Kasumyan and Taufik 1994).

Of the free amino acids, glycine and L-alanine induce food search behaviour in a number of teleost fish species from different systematic and ecological groups. Thus, feeding behaviour is elicited by these substances in Japanese eel (Hashimoto *et al.* 1968), oriental weatherfish (Harada 1985), Atlantic cod (Pawson 1977; Ellingsen and Døving 1986), winter flounder (Sutterlin 1975) and goby (*Gobiosoma bosci*; Hoese and Hoese 1967). However, in many fish species, these two amino acids do not possess a high palatability. In several species, they are inert or act as deterrents; for instance, L-alanine for Siberian sturgeon and grass carp, and glycine for brown trout and European grayling (see Table 7). In the red sea bream, a correlation coefficient with a probability of 0.01 was found between the amino acids that were effective as taste stimuli in bioassays and those that stimulated olfactory neural activity (Goh and Tamura 1980a,b).

Natural deterrents

There are numerous aquatic plants and animals that use chemical defence as protection against fish. These chemicals might be toxic, venomous or deterrent compounds. Substances isolated from different species of marine invertebrates might be both deterrent and toxic for fish (Thompson *et al.* 1985). However, palatability and toxicity of prey are not necessarily correlated (La Barre *et al.* 1986; Sammarco *et al.* 1987; Wylie and Paul 1988; Sammarco and Coll 1990; Schulte and Bakus 1992). It has been suggested that decrease in palatability might be a more important defensive trait for aquatic prey organisms than toxicity (Schulte and Bakus 1992).

One group of aquatic animals, which are very vulnerable to fish, are sponges. Nevertheless, some sponges are inedible for fish and survive in biotopes like coral reefs, where fish density and the diversity of fish are very high. The palatability of sponge tissue and ability of sponge crude extracts to inhibit fish feeding was investigated in a reef lagoon at Yanutha Island, Mbengga, Fiji Islands. Sponge tissue was cut into small pieces (3–5 mm diameter). The reef fish – the sergent-major, the parrotfish (*Scarus* sp.), the damselfish (*Dascyllus aruanus*) and the green chromis – were fed approximately equal-sized bits of fish meat or bread and then alternately offered pieces of sponge (*Carteriospongia* sp. and *Dysidea* sp.) of similar appearance and dimensions. Bread was also soaked in 100 mL of water into which sponge tissue had been squeezed. Acceptance or rejection of the food items was recorded along with the subsequent behaviour of the fish (Schulte and Bakus 1992). It was found that reef fish consumed all pieces of the bread or fish meat; yet, they never consumed the *Carteriospongia* sp. or *Dysidea* sp. tissue. An exception was one trial in which 50–70% of the bread that had been soaked in seawater containing aqueous extract of *Carteriospongia* sp. was consumed and only 0–38% of the bread soaked in *Dysidea* sp. extract was consumed. These results show that the sponge tissue contained substance(s) that have deterrent effect on reef fish and prevent sponges from being eaten by fish. Bakus *et al.* (1989) showed that all exposed benthic, soft-bodied, macrobiota tested in the Fiji (22 species) and Maldives (17 species) Islands were unpalatable to resident coral reef fish. In a survey of Caribbean sponges, crude extract from 44 of 71 species exhibited properties that deterred fish feeding (Pawlik *et al.* 1995).

Besides sponges, various other aquatic organisms use deterrents to protect themselves against predators. Addition of live nemertides or polychaetes to tank water with Dover sole does not induce any obvious response. If, however, the head of a Dover sole happens to come in contact with either of these animals, the fish react with a violent avoidance response. The Dover sole rapidly swims away, 'shaking' or 'nodding' its head. The addition of polychaete

© 2003 Blackwell Publishing Ltd,

mucus in the diet as well as saponins (glycosids) isolated from blue starfish also reduces the food consumed (Mackie and Mitchell 1982b). Various corals (Mackie 1987; Fenical and Pawlik 1991; Kerr and Paul 1995; Epifanio *et al.* 1999b), green algae and cyanobacteria (Paul *et al.* 1993; Thacker *et al.* 1997), nudibranchs (Rogers and Paul 1991), nemertean (Heine *et al.* 1991), common oceanic holoplankton organisms (McClintock *et al.* 1996), echinoderms (Bryan *et al.* 1997), gastropods (McClintock and Janssen 1990), aquatic bugs, mites and beetles (Kerfoot *et al.* 1980; Gerhart *et al.* 1991; Lokensgard *et al.* 1993), polychaete worms (Prezant 1980; Yoshiyama and Darling 1982), prey fish (Winn and Bardach 1959; Gladstone 1987), eggs, hatchlings and tadpoles of anurans (Voris and Bacon 1966; Kruse and Francis 1977; Kats *et al.* 1988; Komak and Crossland 2000; Crossland 2001) and other groups of aquatic organisms use chemical deterrent to make themselves inedible for fish. Duffy and Paul (1992) suggested that chemical defences are less effective in high- than in low-quality foods.

The existence of chemical defence mechanisms in food organisms is a widely recognised phenomenon. Unfortunately, few of the deterrent substances have been identified as the research on such chemical defences has only recently begun. Many of the unusual secondary metabolites that have been isolated from soft-bodied marine invertebrates and marine weeds are proposed to function as agents that deter predation (for review, see Paul 1992; Pawlik 1993). Some deterrents have been identified as saponins (Lucas *et al.* 1979), polyketide sulphates (Rideout *et al.* 1979), terpenoids, acetogenins, unusual fatty acids, phlorotannins, malyngogamides (Hay 1991; Thacker *et al.* 1997) and steroids (pregnanes; Gerhart *et al.* 1991; Lokensgard *et al.* 1993).

A new polymeric pyridinium alkaloid, named amphitoxin, has been isolated as the chemical defence of the Caribbean sponge *Amphimedon compressa* (Albrizio *et al.* 1995). Two compounds, an alkaloid called oroidin and its carboxypyrrole hydrolysis product, 4,5-dibromopyrrol-2-carboxylic acid, have been identified as primary defensive agents of Caribbean sponges of the genus *Agelas* against the reef fish, bluehead wrasse (Channas *et al.* 1996). Recently, dibrominated alkaloid stevensine, closely related to the compound oroidin, has been isolated from the Caribbean sponge *Axinella corrugata*. A crude extract of the sponge, which includes a butanol-soluble partition of the extract, deterred feeding of the bluehead wrasse in laboratory assays so long as the extract had the same concentration as in the natural state. Stevensine also deterred feeding in a wide assemblage of fish in assays performed on reefs where *A. corrugata* is found (Wilson *et al.* 1999). Furanosesterpene variabilin, an ichthyodeterrent, has been isolated from the sponge *Ircinia strobilina* (Epifanio *et al.* 1999a). A medium ring haloether, brasilene, has been isolated from the opisthobranch, and this compound acts as a feeding deterrent against several species of fish (Kinnel *et al.* 1979). Concentrations of secondary metabolites in prey tissue can be quite high, for example, scalaradial constituted 2.4% of the total dry mass in the Pacific sponge (Rogers and Paul 1991). Concentration of stevensine in the Caribbean sponge *A.* corrugata, ranged from 12 to 30 mg mL^{-1} sponge tissue (mean 19.0 mg mL^{-1}; Wilson *et al.* 1999).

A well-known neurotoxin found in the skin of puffer fish species, tetrodotoxin, also has deterrent properties. Behavioural studies demonstrated that some fish species reject toxic puffer tissues and artificial food pellets containing tetrodotoxin (Yamamori *et al.* 1980, cited in Saito *et al.* 1984; Yamamori *et al.* 1988). Electrophysiological experiments in rainbow trout and Arctic char demonstrated that tetrodotoxin was a highly effective taste stimulus (Yamamori *et al.* 1988). Aquarium observations in which Dusky grouper was kept in starved condition showed that they swallowed live mice, but promptly rejected small puffer fish (Freitas *et al.* 1992). The fertilised eggs and newly hatched larvae of a tetraodontid, such as sharpnose puffer fish, are unpalatable to potential egg predators. In experiment, both eggs and larvae were mouthed, then immediately rejected by wrasse (*Thalassoma lunare*), and damselfish (*Dascyllus reticulates*). It was suggested that tetrodotoxin is present in skin, liver, intestine and ovaries of tetraodontids, thus making the eggs and larvae of these fish unpalatable. The nonpalatability of their fertilised eggs probably explains the absence of parental care in these domersal spawning species (Gladstone 1987).

Chemical defence among prey organisms is especially important in tropical coral reefs, where fish may forage the bottom using in excess of 150 000 bites m^{-2} day^{-1}, and where either fish or sea-urchins alone are capable of removing nearly 100% of the daily production (Carpenter 1986). The dominance of certain coral reef species may result in part from their unpalatability to fish. Fish-feeding experiments on coral reefs showed that all exposed soft-bodied benthic macrobiota animals were unpa-

latable and rejected by a variety of predators (Bakus *et al.* 1989). More than half of the common coral reef invertebrates are toxic to fish (Bakus 1981). It was found that fish of the temperate zone might be more affected by seaweed deterrents then the tropical herbivorous fish (Cronin *et al.* 1997). It was also shown that the combination of secondary metabolites and calcium carbonate in the aragonite form proved a more effective deterrent than either secondary metabolites or aragonite alone (Meyer and Paul 1995).

It seems to be a general feature that fish stimulants are species-specific, thus the spectra of stimulants have different composition for different species. This finding is in conspicuous contrast to the finding that deterrents, like secondary metabolites, are not species-specific, but are highly efficient for a range of fish species. For example, a crude extract of sponge (*Axinella corrugata*) at the same concentration as in the sponge tissue significantly deterred feeding in a variety of fish species present on the reef where the sponge inhabited. They were wrasses (*Thalassoma* spp.) and *Halichoeres* spp., snapper, parrotfish (*Scarus* spp.) and *Sparisoma* spp., grunts, tilefish, porgy and angelfish (*Pomacanthus* spp.) and *Holacanthus* spp. (Wilson *et al.* 1999). Moreover, crude extracts of 15 of 17 various coral reef macrobiota in the Fiji and Maldives Islands including sponges, algae, corals, ascidians and sea anemones were unpalatable to goldfish (Bakus *et al.* 1989) that have never been exposed to tropical reefs. Pure secondary metabolites of different species of marine demosponges from San Diego, California inhibited feeding of goldfish as well as feeding of several species inhabiting the same area where demosponges were collected (Thompson *et al.* 1985).

Application in aquaculture and fisheries

Information about the palatability of feeding substances provides a basic knowledge in fish physiology, but such information might be of significant value when applied to cultivation and fisheries. The growing use of fish in aquaculture demands sources for less expensive food for the fish. The use of additives that improve the palatability of artificial food for fish is a probable application. The possible destruction of fish biotopes by trawling could promote and enforce less damaging fishing methods, such as passive fishing gears like traps and long lines. These fishing methods have been used for centuries and are not outdated. Their use, however, requires refinements that use substances that attract the fish from a distance and substances that have the correct palatability for the fish species that preferably should be caught. The artificial baits have begun their era in sport fishing, and the time is ripe for their use in commercial fisheries.

Knowledge of feeding behaviour of fish in aquaculture is important so that feeds and feeding techniques can be designed to encourage consumption and hence survival and growth whilst minimising metabolic energy expenditure in feeding (Jobling 1994). The eventual incorporation of feeding stimulants into diets consisting of cheap and normally non-palatable protein sources may be of practical significance to the fish farming industry (Jobling *et al.* 2001). Furthermore, wastage must be minimised because of high feed costs and the potentially deleterious effects of waste food on water quality. Non-acceptance of artificial food, which can cause large fish mortality, is a serious problem in aquaculture, especially in the rearing of marine fish larvae (de la Higuera 2001). Large amounts of free amino acids, which have high solubility in water, were lost during mastication by fish. For common carp, the percentage loss of amino acids within 15 min after feeding reaches more then 70% for arginine, leucine, isoleucine, lysine, methionine, valine, alanine, glutamic acid, glycine, proline, serine and some others (Yamada and Yone 1986).

There are several publications that show the importance of introducing taste stimulants into modern fish cultivation technologies. It was shown that start-feeding of European glass eels with a trout fry crumble can be improved significantly by adding a mixture of the amino acids L-alanine, glycine, L-histidine and L-proline to the feed (Kamstra and Heinsbroek 1991). The mixture used as attractant was based on the mixture applied by Takii *et al.* (1984). In this mixture, the authors also used a nucleotide, uridine-5′-monophosphate (UMP), which had a small synergistic effect. In the experiments of Kamstra and Heinsbroek 1991), UMP was omitted from the mixture as it was so costly that it would prevent practical application. Addition of amino acid mixture significantly increased the *ad libitum* metabolizable energy of the diets from 45 kJ $kg^{-0.8}$ day^{-1} to 50-60 kJ $kg^{-0.8}$ day^{-1}, which resulted in an increase in growth rate from 1.3 g $kg^{-0.8}$ day^{-1} to 2.4 g $kg^{-0.8}$ day^{-1} (Heinsbroek and Kreuger 1992). Using a mixture that stimulates feeding, which contained L-alanine, L-serine, inosine-5′-monophosphate and betaine in plant-based diets, improved

 © 2003 Blackwell Publishing Ltd,

feed acceptability and growth performance of cultured striped bass, fed low-cost, plant-based diets (Papatryphon and Soares 2000). Improvements in feed intake, weight gain and feed efficiency as a result of feeding stimulant supplementation have been previously observed in gilthead sea bream (Tandler *et al.* 1982) and European sea bass (Dias *et al.* 1997).

Another example showing the significance of taste qualities in artificial fish feed has been shown in the results obtained by Stradmeyer (1989). It was found that just after neutralising the salmon feed (Frippak Feeds Ltd.; pH 3–4) pellet ingestion by Atlantic salmon fry increased by a factor of two in comparison to non-neutralised food. It has been shown that within the first minute of feeding, fish ate more than 50% of the total feed consumed during the total period (4 min; Oikawa and March 1997). Thus, there is probably an advantage in applying feeding stimulants to the surface of feed pellets, thus minimising the amount required and facilitating its detection by the fish.

It has been reported that fish fed a diet of poor palatability were satiated sooner than fish fed a more acceptable diet. If fish are fed to satiation with a diet of poor palatability and then given a more palatable diet, they will begin eating again until they reach a new satiation level (Ishiwata 1968). It has been shown that artificial food with good chemosensory characteristics is digested by fish more effectively (Takeda and Takii 1992).

Another possible application of feeding stimulants may be to mask deterrent ingredients that may lower the palatability of diets. Unpalatable plant proteins are used as substitutes for fishmeal protein in commercial diets. Certain antibiotics have been shown to reduce the palatability of feed (Schreck and Moffitt 1987; Poe and Wilson 1989; Robinson *et al.* 1990; Hustvedt *et al.* 1991; Robinson and Tucker 1992). Supplement of feeding stimulants might mask deterrent ingredients and improve feed intake (Toften *et al.* 1995; Dias *et al.* 1997; Papatryphon and Soares 2000).

Using special additives might increase palatability of artificial food, but it might also be raised by removing the components with bad taste or by replacing those components with equivalents, which do not provoke negative taste responses. To this end, one must estimate the palatability for each of the components that make up the feed. In one special study, it was shown that components of artificial food have strongly different taste qualities for fish (Kasumyan *et al.* 1995). The palatability of each of the 19 components that make up a feed for 9–12-cm long stellate sturgeon juveniles was tested. The components were fish meal, krill meal, squid meal, meat and bone meal, dry whey, fish protein concentrate, Na-caseinate, yeast hydrolysate, PVF yeast, PVF enzymated yeast, Eprin yeast, coarsely cut sunflower seed, coarsely cut soyabean, wheat flour, wheat bran, fukus grist, PM-2 premix, PF-2V premix and FinnStim (Finnish Sugar Co. Ltd). It was found that water extract of 13 of the components, mainly the fish protein concentrate, PVF enzymated yeast and premixes included in agar-agar pellets provoked the increase of grasp activity in fish. Extracts of PF-2V premix, krill meal, fishmeal concentrate, Eprin yeast and coarsely cut soyabeans significantly increased the pellet consumption. The Japanese pilchard fishmeal stabilised by various antioxidants such as santochin, anfelan-1, anfelan-5 and ionol released different kinds of behavioural taste responses in stellate sturgeon. The FinnStim was ineffective as both oral and extraoral taste stimuli. Based on the results obtained, it was possible to correct the feed formulas so as to improve their palatability for fish (Kasumyan *et al.* 1995).

High protein content and a balanced amino acid profile make the earthworm, one of the most cultivated species in vermiculture, a useful alternative protein source for fish. However, this invertebrate contains unpalatable compounds in the coelomic fluid. The removal of these compounds resulted in an increase in feed intake in rainbow trout fingerlings, and the use of squid extract as a feeding stimulant further improved feed intake of fish fed formulations containing earthworm meal (Cardenete *et al.* 1993).

Epilogue

In this review, we have presented the status for taste preferences in fish, but evidently, what is done so far is only a beginning of an extended series of experiments that will provide a sound scientific basis for our understanding of the subject. In the present review, we have stressed the specificity of taste preferences in fish. We also point out the great variability among individuals. Further, it seems that the fish is indifferent to previous taste experience. This property is probably unexpected among fishers and aquaculturists. It should be noted that the example of patroclinous inheritance of taste preferences does not need to be the same for all species. We hope that

we have made it clear that fish taste is much more than the five modalities which dominate mammalian taste.

Taste preferences are under genetic control, and hence, readily inheritable. The fact that specific classes of substances, such as the amino acids and nucleotides, remain as feeding stimulants across a broad taxonomic expanse of fish orders suggests that the genetic control of taste preference in fish has been phylogenetically conservative. Moreover, there is a strong conservatism of taste preference within species; even those now diverged into different strains and yet there is almost no genetic conservation of taste preference between even closely related species within the same genus having similar diets and occupying similar ecological niches. A possible reason for this apparent contradiction is that we are working with substances that are only of marginal interest to the fish taste receptors. Therefore, it seems a useful aspect for the future to uncover the unknown substances that evidently take action in fish taste preferences. This is needed because there are certainly taste substances that are much more potent than those commonly in use.

A major breakthrough has been the uncovering of the molecular basis for taste receptors. Future studies will undoubtedly uncover the properties of these receptors and which substances the fish can detect. Finally, we would like to add that experiments on fish taste preferences can be made with simple means and a modest budget, and can from someone's viewpoint look like children's play. However, through careful planning and systematic data collection, and by scrutinizing the results, it is possible to obtain important information for science, fisheries and aquaculture.

Acknowledgements

We wish to thank Sergey Sidorov, Abdurahman Kazhlaev, Nikolaj Pashchenko, Amal M. H. Morsy, Ekaterina Nikolaeva, Olga Prokopova and Elena Fokina for their valuable contributions to many of the experiments cited in this review. We are grateful to George Alexander, Paul Hart, Kurt Kotrschal and Kathryn Mearns for helpful comments on an earlier version of our manuscript and for help with the English. This study was supported by the Russian Foundation for the Basic Research, the International Science Foundation, the European Science Foundation and the Norwegian Research Council.

References

Adamek, Z., Fasaic, K. and Debeljak, L. (1990) Lower temperature limits of plant food intake in young grass carp (*Ctenopharyngodon idella* Val.). *Ichthyologia* **22**, 1–8.

Adams, M.A., Johnsen, P.B. and Hong-Qi, Z. (1988) Chemical enhancement of feeding for the herbivorous fish *Tilapia zillii*. *Aquaculture* **72**, 95–107.

Adron, J.W. and Mackie, A.M. (1978) Studies on the chemical nature of feeding stimulants for rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **12**, 303–310.

Albrizio, S., Ciminiello, P., Fattorusso, E., Magno, S. and Pawlik, J.R. (1995) Amphitoxin, a new high molecular weight antifeedant pyridinium salt from the Caribbean sponge *Amphimedon compressa*. *Journal of National Products* **58**, 647–652.

Andriashev, A.P. (1944) Role of sense organs in searching for food in *Gaidropsarus mediterraneus*. *Journal of General Biology (Zhurnal Obshei Biologii)* **5**, 123–127 (in Russian).

Appelbaum, S. (1980) Versuche zur Geschmacksperzeption einiger Susswasserfische im larvalen und adulten Stadium. *Archiv für Fischereiwissenschaft* **31**, 105–114.

Appelbaum, S., Adron, J.W., George, S.G., Mackie, A.M. and Pirie, B.J.S. (1983) On the development of the olfactory and the gustatory organs of the Dover sole, *Solea solea*, during metamorphosis. *Journal of Marine Biological Association, UK* **63**, 97–108.

Atema, J. (1971) Structures and functions of the sense of taste in the catfish (*Ictalurus natalis*). *Brain, Behaviour and Evolution* **4**, 273–294.

Atema, J. (1980) Chemical senses, chemical signals and feeding behaviour in fishes. In: *Fish Behaviour and its Use in the Capture and Culture of Fishes* (eds J.E. Bardach, J.J. Magnuson, R.C. May and J.M. Reinhart). International Center for Living Aquatic Resources Management, Manila, pp. 57–101.

Atema, J., Holland, K. and Ikehara, W. (1980) Olfactory responses of yellowfin tuna (*Thunnus albacares*) to prey odors: chemical search image. *Journal of Chemical Ecology* **6**, 457–465.

Bailey, M. and Sandford, G. (1999) *The Ultimate Encyclopedia of Aquarium Fish and Fish Care*. Lorenz Book, London.

Bakus, G.J. (1981) Chemical defence mechanisms on the Great Barrier Reef, Australia. *Science* **211**, 497–499.

Bakus, G.J., Stebbins, T.D., Lloyd, K. *et al.* (1989) Unpalatability and dominance on coral reefs in the Fiji and Maldive Islands. *Journal of Zoology and Aquatic Biology* **1**, 34–53.

Bannister, L.H. (1974) Possible functions of mucus at gustatory and olfactory systems. In: *Transduction Mechanisms in Chemoreception* (ed. T.M. Poynder). Information Retrieval, London, pp. 39–48.

Bardach, J.E. and Atema, J. (1971) The sense of taste in fishes. In: *Handbook of Sensory Physiology*, **Vol. 4** (ed. L.M. Beider). Springer-Verlag, Berlin, pp. 293–336.

Bardach, J.E., Todd, J.H. and Crickmer, R.K. (1967) Orientation by taste in fish of genus *Ictalurus*. *Science* **155**, 276–1278.

 © 2003 Blackwell Publishing Ltd,

Bardach, J.E., Winn, H.E. and Menzel, D.W. (1959) The role of the senses in the feeding of the nocturnal reef predators *Gymnothorax moringa* and *G. vicinus*. *Copeia* **2**, 133–139.

Barlow, L.A. and Northcutt, R.G. (1995) Embryonic origin of amphibian taste buds. *Developmental Biology* **169**, 273–285.

Beauchamp, G.K., Cowart, B.J. and Mennella, J.A. (1994) Infant salt taste: developmental, methodological, and contextual factors. *Developmental Psychology* **27**, 353–365.

Beauchamp, G.K., Cowart, B.J. and Moran, M. (1986) Developmental changes in salt acceptability in human infants. *Developmental Psychology* **19**, 75–83.

Bernache, L. (2001) The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution* **55**, 351–379.

Boikova, O.S. (1986) Feeding of fish in Lake Glubokoe. *Hydrobiologia* **141**, 95–111.

Brand, J.G. and Bruch, R.C. (1992) Molecular mechanisms of chemosensory transduction: gustation and olfaction. In: *Fish Chemoreception* (ed. T.J. Hara). Chapman & Hall, London, pp. 126–149.

Branson, B.A. (1966) Histological observations on the sturgeon chub, *Hybopsis gelida* (Cyprinidae). *Copeia* **4**, 872–876.

Bridcut, E.E. and Giller, P.S. (1995) Diet variability and foraging strategies in brown trout (*Salmo trutta*): an analysis from subpopulations to individuals. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 2543–2552.

Brown, S.B., Evans, R.E., Thompson, B.E. and Hara, T.J. (1982) Chemoreception and aquatic pollutants. In: *Chemoreception in Fishes* (ed. T.J. Hara). Elsevier, Amsterdam, pp. 363–393.

Bryan, J.E. and Larkin, P.A. (1972) Food specialization by individual trout. *Journal of the Fisheries Research Board of Canada* **29**, 1615–1624.

Bryan, P.J., McClintock, J.B. and Hopkins, T.S. (1997) Sructural and chemical defenses of echinoderms from the nothern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* **210**, 173–186.

Cantoni, G.L. and Anderson, D.G. (1956) Enzymatic cleavage of dimethylpropiothetin by *Polysiphonia lanosa*. *Journal of Biological Chemistry* **222**, 171–177.

Caprio, J. (1975) High sensitivity of catfish taste receptors to amino acids. *Comparative Biochemistry and Physiology* **52A**, 247–251.

Caprio, J. (1978) Olfaction and taste in the channel catfish: an electrophysiological study of the responses to amino acids and derivates. *Journal of Comparative Physiology* **123**, 357–371.

Caprio, J. (1982) High sensitivity and specificity of olfactory and gustatory receptors of catfish to amino acids. In: *Chemoreception in Fishes*. (ed. T.J. Hara). Elsevier Scientific Publishing Co., Amsterdam, pp. 109–134.

Caprio, J. (1984) Olfaction and taste in fish. In: *Comparative Physiology of Sensory Systems. Olfaction and Taste in Fish* (eds L. Bolis, R.D. Keynes and S.H.P. Maddrell). Cambridge University Press, Cambridge, pp. 257–283.

Caprio, J. (1988) Peripheral filters and chemoreceptor cells in fishes. In: *Sensory Biology of Aquatic Animals*. (eds J. Atema, R.R. Fay, A.N. Popper and W.N. Tawolga). Springer-Verlag, Berlin, pp. 313–338.

Cardenete, G., Garzón, A., Moyano, F. and de la Higuera, M. (1993) Nutritive utilization of earthworm protein by fingerling rainbow trout (*Oncorthynchus mykiss*). In: *Fish Nutrition in Practice*. (eds S.J. Kaushik and P. Luquet). INRA Editions, Paris, pp. 923–926.

Carpenter, R.C. (1986) Partitioning herbivory and its effects on coral reef algal communities. *Ecological Monographs* **56**, 345–365.

Carr, W.E.S. (1976) Chemoreception and feeding behaviour in the pigfish, *Orthopristis chrysopterus*: characterization and identification of stimulatory substances in a shrimp extract. *Comparative Biochemistry and Physiology* **55A**, 153–157.

Carr, W.E.S. (1982) Chemical stimulation of feeding behaviour. In: *Chemoreception in Fishes*. (ed. T.J. Hara). Elsevier Scientific Publishing Co., Amsterdam, pp. 259–273.

Carr, W.E.S., Blumenthal, K.M. and Netherton, J.C. III (1977) Chemoreception in the pigfish, *Orthopristis chrysopterus*: the contribution of amino acids and betaine to stimulation of feeding behaviour by various extracts. *Comparative Biochemistry and Physiology* **58A**, 69–73.

Carr, W.E.S. and Chaney, T.B. (1976) Chemical stimulation of feeding behaviour in the pinfish, *Lagodon rhomboides*: characterization and identification of stimulatory substances extracted from shrimp. *Comparative Biochemistry and Physiology* **54A**, 437–441.

Carr, W.E.S., Netherton, J.C. III, Gleeson, R.A. and Derby, C.D. (1996) Stimulants of feeding behavior in fish: analyses of tissues of diverse marine organisms. *Biological Bulletin* **190**, 149–160.

Cembella, A.D., Therriault, J.-C. and Beland, P. (1988) Toxicity of cultured isolates and natural populations of *Protogonyaulax tamarensis* from the St. Lawrence estuary. *Journal of Shellfish Ressearch* **7**, 611–621.

Challenger, F. and Simpson, M.I. (1948) Studies on biological methylation. Part XII. A precursor of the dimethylnsulphide evolved by *Polysiphonia fastigata*. Dimethyl-2-carboxyethylsulphonium hydroxide and its salts. *Journal of Chemical Society* **30**, 1591–1597.

Channas, B., Pawlik, J.R., Lindel, T. and Fenical, W. (1996) Chemical defense of the Carribbean sponge *Agelas clathrodes* (Schmidt). *Journal of Experimental Marine Biology and Ecology* **208**, 185–196.

Colgan, P.W., Brown, J.A. and Orsatti, S.D. (1986) Role of diet and experience in the development of feeding behaviour in largemouth bass, *Micropterus salmoides*. *Journal of Fish Biology* **28**, 161–170.

Coughlin, D.J. and Strickler, J.R. (1990) Zooplankton capture by a coral reef fish: an adaptive response to evasive prey. *Environmental Biology of Fishes* **29**, 35–42.

Cronin, G., Paul, V.J., Hay, M.E. and Fenical, W. (1997) Are tropical herbivores more resistant than temperate herbivores to seaweed chemical defenses? Ditepenoid metabolites from Dictyota acutiloba as feeding deterrents for tropical versus temperate fishes and urchins. *Journal of Chemical Ecology* **23**, 289–302.

Crossland, M.R. (2001) Ability of predatory native Australian fishes to learn to avoid toxic larvae of the introduced toad, *Bufo marinus*. *Journal of Fish Biology* **59**, 319–329.

Dabrowski, K. and Rusiecki, M. (1983) Content of total and free amino acids in zooplanktonic food of fish larvae. *Aquaculture* **30**, 31–42.

Davenport, C.J. and Caprio, J. (1982) Taste and tactile recording from the ramus recurrens facialis innervating flank taste buds in the catfish. *Journal of Comparative Physiology* **147A**, 217–229.

De la Noue, J. and Choubert, G. (1985) Apparent digestibility of invertebrate biomasses by rainbow trout. *Aquaculture* **50**, 103–112.

Devitsina, G.V. and Kazhlaev, A.A. (1993) Development of chemosensory organs in Siberian sturgeon, *Acipenser baeri*, and stellate sturgeon, *A. stellatus*. *Journal of Ichthyology* **33**, 9–19.

Devitsina, G.V. and Kazhlaev, A.A. (1995) Chemosensory systems and their heterohronic morphogenesis in sturgeons. *Biofizika* **40**, 146–150.

Dias, J., Gomes, E.F. and Kaushik, S.J. (1997) Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets. *Aquatic Living Resources* **10**, 385–389.

Døving, K.B. (1986) Functional properties of the fish olfactory system. In: *Progress in Sensory Physiology*, **Vol. 6**. Springer Verlag, Berlin e.a, pp. 39–104.

Duffy, J.E. and Paul, V.J. (1992) Prey nutritional quality and the effectiveness of chemical defenses against tropical reef. *Oecologia* **90**, 333–339.

Dussault, G.V. and Kramer, D.L. (1981) Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Canadian Journal of Zoology* **59**, 684–701.

Ellingsen, O.F. and Døving, K.B. (1986) Chemical fractionation of shrimp extracts inducing bottom food search behavior in cod (*Gadus morhua* L.). *Journal of Chemical Ecology* **12**, 155–168.

Epifanio, R.D.A., Gabriel, R., Martins, D.L. and Muricy, G. (1999a) The sesterpene variabilin as a fish-predation deterrent in the Western Atlantic sponge *Ircinia strobilina*. *Journal of Chemical Ecology* **25**, 2247–2254.

Epifanio, R.D.A., Martins, D.L., Villaca, R. and Gabriel, R. (1999b) Chemical defences against fish predation in three Brazilian octacorals: 11β,12β-epoxypukalide as a feeding deterrent in *Phyllogorgia dilatuta*. *Journal of Chemical Ecology* **25**, 2255–2265.

Farr, J.A. and Herrnkind, W.F. (1974) A quantitative analysis of social interaction of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae) as function of population density. *Animal Behaviour* **22**, 582–591.

Fenical, W. and Pawlik, J.R. (1991) Defensive properties of secondary metabolites from the Carribbean gorgonian coral *Erythropodium caribaeorum*. *Marine Ecology Progress in Series* **75**, 1–8.

Finger, T.E. (1976) Gustatory pathways in the bullhead catfish. Part I. Connections of the anterior ganglion. *Journal of Comparative Neurology* **165**, 513–526.

Finger, T.E., Drake, S.K., Kotrschal, K., Womble, M. and Dockstader, K.C. (1991) Postlarval growth of the peripheral gustatory system in the channel catfish, *Ictalurus punctatus*. *Journal of Comparative Neurology* **314**, 55–66.

Finger, T.E. and Morita, Y. (1985) Two gustatory systems: facial and vagal gustatory nuclei have different brainstem connections. *Science* **227**, 776–778.

Fischer, Z. (1973) The elements of energy balance in grass carp (*Ctenopharyngodon idella* Val.). Part IV. Consumption rate of grass carp fed on different types of food. *Polish Archives of Hydrobiology* **20**, 309–318.

Flerova, G.I. and Gdovskii, P.A. (1976) The rate of excitement propagation in olfactory nerve fibres and responses in the smell brain of fishes under varied temperature conditions. *Voprosi Ikhtiologii* **16**, 119–125 (in Russian).

Formaker, B.K. and Hill, D.L. (1990) Alterations of salt taste perception in the developing rats. *Behavioral Neuroscience* **104**, 356–364.

Fowler, K.L., Pauley, G.B. and Thomas, G.L. (1978) The effects of the food preferences and stocking rates of grass carp (*Ctenopharyngodon idella* Val.) on mixed plant communities. *Aqautic Botany* **5**, 261–276.

Franco, J., Johnstone, A.D.F. and Mackie, A.M. (1991) Studies of bait preference in the cod *Gadus morhua* L. Characterisation of feeding stimulants using an operant conditioning technique. *Fisheries Research* **10**, 229–242.

Freitas, J.C., Rantin, F.T., Nonaka, K.O. and Almeida, F.E.X. (1992) Bradycardia in groupers (*Epinephelus guaza*) induced by taste stimuli from puffer fish (*Sphaeroides spengleri*) skin secretion. In: *Recent Advances in Toxicology Research. Bradycardia in Groupers (Epinephelus Guaza) Induced by Taste Stimuli from Puffer Fish (Sphaeroides Spengleri) Skin Secretion*, Vol. 2 (eds P. Gopalakrishnakone and C.K. Tan). National University of Singapore, Singapore, pp. 465–475.

Fryer, G. and Iles, T.D. (1972) *The Cichlid Fishes of the Great Lakes of Africa: the Biology and Evolution*. Oliver and Boyd, Edinburgh.

Fuke, S., Konosu, S. and Ina, K. (1981) Identification of feeding stimulants for red sea bream in the extract of marine worm *Perineries brevicirrus*. *Bulletin of the Japanese Society of Scientific Fisheries* **47**, 1631–1635.

Gerhart, D.J., Bondura, M.E. and Commito, J.A. (1991) Inhibition of sunfish feeding by defensive steroids from aquatic

 © 2003 Blackwell Publishing Ltd,

beetles: structure-activity relationships. *Journal of Chemical Ecology* **17**, 1363–1370.

Gladstone, W. (1987) The eggs and larvae of the sharpnose pufferfish *Canthigaster valentini* (Pisces, Tetraodontidae) are unpalatable to other reef fishes. *Copeia* **1**, 227–230.

Glaser, D. (1966) Untersuchungen über die absoluten Geschmacksschwellen von Fischen. *Zeitschrift für Vergleichende Physiologie* **52**, 1–25.

Goh, Y. and Tamura, T. (1980a) Effect of amino acids on the feeding behaviour in red sea bream. *Comparative Biochemistry and Physiology* **66C**, 225–229.

Goh, Y. and Tamura, T. (1980b) Olfactory and gustatory responses to amino acids in two marine teleosts – red sea bream and mullet. *Comparative Biochemistry and Physiology* **66C**, 217–224.

Gomahr, A., Palzenberger, M. and Kotrschal, K. (1992) Density and distribution of external taste buds in cyprinids. *Environmental Biology of Fishes* **33**, 125–134.

Gosselin, S., Fortier, L. and Gagne, J.A. (1989) Vulnerability of marine fish larvae to the toxic diniflagellate *Protogonyaulax tamarensis*. *Marine Ecology Progress in Series* **57**, 1–10.

Grobecker, D.B. and Pietsch, T.W. (1979) High-speed cinematographic evidence for ultrafast feeding in Atennariid anglerfishes. *Science* **205**, 1161–1162.

Hansen, A., Reutter, K. and Zeiske, E. (2002) Taste bud development in the zebrafish, *Danio rerio*. *Developmental Dynamics* **223**, 483–496.

Hara, T.J. (1994a) The diversity of chemical stimulation of fish olfaction and gustation. *Reviews in Fish Biology and Fisheries* **4**, 1–35.

Hara, T.J. (1994b) Olfaction and gustation: an overview. *Acta Physiologica Scandinavica* **152**, 207–217.

Hara, T.J., Carolsfeld, J. and Kitamura, S. (1999) The variability of the gustatory sensibility in salmonids, with special reference to strain differences in rainbow trout, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 13–24.

Hara, T.J. and Marui, T. (1984) Multiplicity of taste receptors for amino acids in rainbow trout: evidence from cross-adaptation and kinetic analysis. *Chemical Senses* **8**, 250.

Hara, T.J., Sveinsson, T., Evans, R.E. and Klaprat, D.A. (1993) Morphological and functional characteristics of the olfactory and gustatory organs of three Salvelinus species. *Canadian Journal of Zoology* **71**, 414–423.

Harada, K. (1985) Feeding attraction activities of amino acids and nitrogeneous bases for oriental weatherfish. *Bulletin of the Japanese Society of Scientific Fisheries* **51**, 461–466.

Harborne, J.B. (1993) *Introduction to Ecological Biochemistry*, 4th edn. Academic Press, London.

Hashimoto, Y., Konosu, S., Fusetani, N. and Nose, T. (1968) Attractants for eels in the extracts of short-necked clam. Part I. Survey of constituents eliciting feeding behaviour by the omission test. *Bulletin of the Japanese Society of Scientific Fisheries* **34**, 78–83.

Hay, M.E. (1991) Fish–seaweed interactions on coral reefs: effects of herbivorous fishes and adaptations of their prey. In: *The Ecology of Fishes on Coral Reefs*. (ed. P.F. Sale). Academic Press, San Diego, pp. 96–119.

Hayama, T. and Caprio, J. (1989) Lobule structure and somatotopic organization of the medullary facial lobe in the channel catfish, *Ictalurus punctatus*. *Journal of Comparative Neurology* **285**, 9–17.

Hayama, T. and Caprio, J. (1990) Somatotopical organization of the intermediate nucleus of the facial lobe in the channel catfish, *Ictalurus punctatus*. *Brain Research* **516**, 137–140.

Heine, J.N., McClintock, J.B., Slattery, M. and Weston, J. (1991) Energetic composition, biomass and chemical defense in the common antarctic nemertean *Parborlasia corrugatus* McIntosh. *Journal of Experimental Marine Biology and Ecology* **153**, 15–25.

Heinsbroek, L.T.N. and Kreuger, J.G. (1992) Feeding and growth of glass eels, *Anguilla anguilla* L. The effect of feeding stimulants on feed intake, energy metabolism and growth. *Aquaculture and Fisheries Management* **23**, 327–336.

Herrick, C.J. (1901) The cranial nerves and cutaneous sense organs of the North American silurid fishes. *Journal of Comparative Neurology and Psychology* **11**, 177–249.

Hidaka, I. (1982) Taste receptor stimulation and feeding behavior in the puffer. In: *Chemoreception in Fishes*. (ed. T.J. Hara). Elsevier Scientific Publishing Co., Amsterdam, pp. 243–257.

Hidaka, I. and Ishida, Y. (1985) Gustatory response in the Shimaisaki (tigerfish) *Therapon oxyrhynchus*. *Bulletin of the Japanese Society of Scientific Fisheries* **51**, 387–391.

Hidaka, I., Kohbara, J., Araki, T., Morishita, T., Miyajima, T., Shimizu, S. and Kuriyama, I. (2000) Identification of feeding stimulants from a jack mackerel muscle extract for young yellowtail *Seriola quinqueradiata*. *Aquaculture* **181**, 115–126.

Hidaka, I., Kyiohara, S. and Oda, S. (1977) Gustatory response in the puffer. Part III. Stimulatory effectiveness of nucleotides and their derivatives. *Bulletin of the Japanese Society of Scientific Fisheries* **43**, 423–428.

Hidaka, I., Nyu, N. and Kiyohara, S. (1976) Gustatory response in the puffer. Part IV. Effects of mixtures of amino acids and betaine. *Bulletin of the Faculty of Fisheries, Mie University* **3**, 17–28.

Hidaka, I., Ohsugi, T. and Kubomatsu, T. (1978) Taste receptor stimulation and feeding behaviour in the puffer Fugu pardalis. Part I. Effect of single chemicals. *Chemical Sense and Flavor* **3**, 341–354.

de la Higuera, M. (2001) Effects of nutritional factors and feed characteristics on feed intake. In: *Food Intake in Fish*. (eds D. Houlihan, T. Boujard and M. Jobling). Blackwell Science, Oxford, pp. 250–268.

Hoagland, H. (1933) Specific nerve impulses from gustatory and tactile receptors in catfish. *Journal of General Physiology* **16**, 685–693.

Hoese, H.D. and Hoese, D. (1967) Studies on the biology of the feeding reaction in *Gobiosoma bosci*. *Tulane Studies in Zoology* **14**, 55–62.

Holm, J.C. and Walter, B. (1988) Free amino acids in live freshwater zooplankton and dry feed: possible importance for first feeding in Atlantic salmon fry (*Salmo salar*). *Aquaculture* **71**, 341–354.

Hoogerhoud, R.J.C. (1989) Second International Symposium Vertebr. Morphol. Stuttgart, pp. 19–21.

Horppila, J., Ruuhijarvi, J., Rask, M., Karppinen, C., Nyberg, K. and Olin, M. (2000) Seasonal changes in the diets and relative abundances of perch and roach in the littoral and pelagic zones of a large lake. *Journal of Fish Biology* **56**, 51–72.

Hughes, S.G. (1991) Response of first-feeding spring chinook salmon to four potential chemical modifiers of feed intake. *Progressive Fish-Culturist* **53**, 15–17.

Hughes, S.G. (1993) Single-feeding response of chinook salmon fry to potential feed intake modifiers. *Progressive Fish-Culturist* **55**, 40–42.

Hunter, J.R. (1980) The feeding behaviour and ecology of marine fish larvae. In: *Fish behaviour and its use in the capture and calture of fishes*. International Center for Living Aquatic Resources Management, Manila, pp. 287–330.

Hustvedt, S.O., Storebakken, T. and Salte, R. (1991) Does oral administration of oxolinic acid or tetracycline affect feed intake of rainbow trout? *Aquaculture* **92**, 109–113.

Iida, H., Nakamura, K. and Tokunaga, T. (1985) Dimethyl sulfide and dimethyl-β-propiothetin in sea algae. *Bulletin of the Japanese Society of Scientific Fisheries* **51**, 1145–1150 (in Japanese).

Iida, H., Nakamura, K. and Tokunaga, T. (1992) Non-protein nitrogenous compounds in muscle extract of oceanic cephalopods. *Nippon Suisan Gakkaishi* **58**, 2383–2390.

Iida, H. and Tokunaga, T. (1986) Dimethyl sulfide and dimethyl-β-propiothetin in shellfish. *Bulletin of the Japanese Society of Scientific Fisheries* **52**, 557–563.

Ikeda, I., Hosokawa, H., Shimeno, S. and Takeda, M. (1988a) Identification of feeding stimulant for jack mackerel in its muscle extract. *Bulletin of the Japanese Society of Scientific Fisheries* **54**, 229–233.

Ikeda, I., Hosokawa, H., Shimeno, S. and Takeda, M. (1988b) Identification of feeding stimulant in the krill extract for jack mackerel. *Bulletin of the Japanese Society of Scientific Fisheries* **54**, 235–238.

Ikeda, I., Hosokawa, H., Shimeno, S. and Takeda, M. (1991) Feeding stimulant activity of nucleotides, tryptophan and their related compounds for jack mackerel. *Nippon Suisan Gakkashi* **57**, 1539–1542.

Ishida, Y. and Hidaka, I. (1987) Gustatory response profiles for amino acids, glycinebetaine and nucliotides in several marine teleosts. *Bulletin of the Japanese Society of Scientific Fisheries* **53**, 1391–1398.

Ishiwata, N. (1968) Ecological studies on the feeding of fishes. Part VI. External factors affecting satiation amount. *Bulletin of the Japanese Society of Scientific Fisheries* **34**, 785–791.

Ivlev, V.S. (1961) *Experimental Ecology of the Feeding of Fishes*. Yale University Press, New Haven.

Jakubowski, M. (1983) New details of the ultrastructure (TEM, SEM) of taste buds in fishes. *Zeitschrift für Mikroskopish-Anatomische Forschung* **97**, 849–862.

Jakubowski, M. and Whitear, M. (1990) Comparative morphology and cytology of taste buds in teleosts. *Zeitschrift für Mikroskopish-Anatomische Forschung* **104**, 529–560.

Jobling, M. (1994) *Fish Bioenergetics*. Chapman & Hall, London.

Jobling, M., Arnesen, A.M., Baardvik, B.M., Christiansen, J.S. and Jørgensen, E.H. (1995) Monitoring feeding behaviour and food intake: methods and applications. *Aquaculture Nutrition* **1**, 131–143.

Jobling, M., Gomes, E. and Dias, J. (2001) Feed types, manufacture and ingredients. In: *Food Intake in Fish*. (eds D. Houlihan, T. Boujard and M. Jobling). Blackwell Science, Oxford, pp. 25–48.

Johnsen, P.B. and Adams, M.F. (1986) Chemical feeding stimulants for the herbivorous fish, *Tilapia zillii*. *Comparative Biochemistry and Physiology* **83A**, 109–112.

Johnstone, A.D.F. and Mackie, A.M. (1990) Laboratory investigations of bait acceptance by the cod *Gadus morhua* L. Identification of feeding stimulants. *Fisheries Research* **9**, 219–230.

Jones, K.A. (1980) Temperature dependent attraction by goldfish to a chemical feeding cue presented alone and in combination with heated water. *Physiology and Behavior* **33**, 509–515.

Jones, K.A. (1983) Effects of temperature on the activity and orientation of goldfish (*Carassius auratus*). *Comparative Biochemistry and Physiology* **74A**, 777–780.

Jones, K.A. (1989) The palatability of amino acids and related compounds to rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* **34**, 149–160.

Jones, K.A. (1990) Chemical requirements of feeding in rainbow trout *Oncorhynchus mykiss* (Walbaum): palatability studies on amino acids, amides, amines, alcohols, aldehydes, saccharides, and other compounds. *Journal of Fish Biology* **37**, 413–423.

Kaku, T., Tsumagari, M., Kiyohara, S. and Yamashita (1980) Gustatory responses in the minnow, *Pseudorasbora parva*. *Physiology and Behavior* **25**, 99–105.

Kamstra, A. and Heinsbroek, L.T.N. (1991) Effects of attractants on start of feeding of glass eel, *Anguilla anguilla* L. *Aquaculture and Fisheries Management* **22**, 47–56.

Kanwal, J.S. and Caprio, J. (1983) An electriphysiological investigation of the oropharyngeal (IX–X) taste system in the channel catfish, *Ictalurus punctatus*. *Journal of Comparative Physiology* **150A**, 345–357.

Kanwal, J.S. and Caprio, J. (1988) Overlapping taste and tactile maps of the oropharynx in the vagal lobe of the channel catfish, *Ictalurus punctatus*. *Journal of Neurobiology* **19**, 211–222.

 © 2003 Blackwell Publishing Ltd,

Kanwal, J.S. and Finger, T.E. (1992) Central representation and projections of gustatory systems. In: *Fish Chemoreception*. (ed. T.J. Hara). Chapman & Hall, London, pp. 79–102.

Kapoor, B.G., Evans, H.E. and Pevzner, R.A. (1975) The gustatory system in fish. *Advances in Marine Biology* **13**, 53–108.

Kasahara, K. and Nishibori, K. (1987) Effect of heating temperature on volatiles of roasted laver. *Nippon Suisan Gakkaishi* **53**, 673–676 (in Japanese).

Kassil, V.G. (1972) Taste. In: *Physiology of Sensory System*, **Vol. 2**. Nauka, Leningrad, pp. 562–606 (in Russian).

Kasumyan, A.O. (1992) In: *Chemical Signals in Vertebrates VI* (eds R.L. Doty and D. Muller-Schwarze). Plenum Press, New York, Philadelphia, pp. 135–139.

Kasumyan, A.O. (1994) Olfactory sensitivity of the sturgeon to free amino acids. *Biophysics* **39**, 519–522.

Kasumyan, A.O. (1997) Gustatory reception and feeding behavior in fish. *Journal of Ichthyology* **37**, 72–86.

Kasumyan, A.O. (1999a) Olfaction and taste in sturgeon behaviour. *Journal of Applied Ichthyology* **15**, 228–232.

Kasumyan, A.O. (1999b) Olfaction, extraoral and oral taste senses have different level of stability in fish phylogeny. *Chemical Senses* **24**, 530.

Kasumyan, A.O. (2000) Individual variability of taste preferences and of behavioral response to taste stimuli in carp *Cyprinus carpio*. *Journal of Ichthyology* **40**, 661–669.

Kasumyan, A.O. and Devitsina, G.V. (1997) The effect of olfactory deprivation on chemosensory sensitivity and the state of taste receptors of Acipenserids. *Journal of Ichthyology* **39**, 786–798.

Kasumyan, A.O. and Kazhlaev, A.A. (1993a) Behavioral responses of early juveniles of Siberian sturgeon, *Acipenser baeri*, and stellate sturgeon *A. stellatus* (Acipenseridae) to gustatory stimulating substances. *Journal of Ichthyology* **33**, 85–97.

Kasumyan, A.O. and Kazhlaev, A.A. (1993b) Formation of searching behavioral reaction and olfactory sensitivity to food chemical signals during ontogeny of sturgeons (Acipenseridae). *Journal of Ichthyology* **33**, 51–65.

Kasumyan, A.O. and Morsy, A.M.H. (1996) Taste sensitivity of common carp *Cyprinus carpio* to free amino acids and classical taste substances. *Journal of Ichthyology* **36**, 391–403.

Kasumyan, A.O. and Morsy, A.M.H. (1997) Taste preference for classic taste substances in juveniles of the grass carp *Ctenopharyngodon idella* (Cyprinidae, Pisces) reared on various diets. *Doklady Biological Sciences* **357**, 284–286.

Kasumyan, A.O. and Morsy, A.M.H. (1998a) Effect of heavy metals on the feeding activity and taste responses of carp *Cyprinus carpio*. Copper, cadmium, zinc and lead. *Journal of Ichthyology* **38**, 393–409.

Kasumyan, A.O. and Morsy, A.M.H. (1998b) *VIII Intern Symposium on Fish Physiology*. Uppsala University, Uppsala, Sweden, 101 pp.

Kasumyan, A.O. and Nikolaeva, E.V. (1997) Taste preferences of *Poecilia reticulata* (Cyprinidontiformes). *Journal of Ichthyology* **37**, 662–669.

Kasumyan, A.O. and Nikolaeva, E.V. (2002) The comparative analysis of taste preferences in fish with different ecology and feeding. *Journal of Ichthyology* **42**, 203–214.

Kasumyan, A.O. and Ponomarev, V.Y. (1986) Study of the behaviour of zebrafish, *Brachidanio rerio*, in response to natural chemical food stimuli. *Journal of Ichthyology* **26**, 96–105.

Kasumyan, A.O. and Ponomarev, V.Y. (1989) *Role of Chemoreception in Feeding Behavior of Cyprinids. Chemosensitivity and Chemocommunication in Fishes*. Nauka, Moscow, pp. 123–131 (in Russian).

Kasumyan, A.O. and Prokopova, O.M. (2001) Taste preferences and the dynamics of behavioral taste response in the tench, *Tinca tinca* (Cyprinidae). *Journal of Ichthyology* **41**, 640–653.

Kasumyan, A.O. and Sidorov, S.S. (1993a) Behavioral responses of caspian brown trout parr, *Salmo trutta caspius* Kessler, to the main types of taste substances. *Moscow University Biological Sciences Bulletin* **48**, 48–53.

Kasumyan, A.O. and Sidorov, S.S. (1993b) Taste sensitivity of chum salmon, *Oncorhynchus keta*, to types of taste stimuli and amino acids. *Sensory Systems* **6**, 222–225.

Kasumyan, A.O. and Sidorov, S.S. (1994) Comparison of intra- and extra-oral taste responses to free amino acids in three sturgeon species of the genus Acipenser. *Biophysics* **39**, 523–526.

Kasumyan, A.O. and Sidorov, S.S. (1995a) A comparative analysis of the taste responses of young salmon trout, *Salmo trutta trutta*, from populations of the Baltic and White seas. *Doklady Biological Sciences* **343**, 289–391.

Kasumyan, A.O. and Sidorov, S.S. (1995b) *Environmental Science and Vulnerable Ecosystems*. 5th SETAC-Europe Congress, Copenhagen, 284 pp.

Kasumyan, A.O. and Sidorov, S.S. (1995c) Gustatory properties of free amino acids in Caspian trout, *Salmo trutta caspius*. *Journal of Ichthyology* **35**, 8–20.

Kasumyan, A.O. and Sidorov, S.S. (1995d) The palatability of free amino acids and classical taste substances to frolich charr, *Salvelinus alpinus erythrinus* (Georgi). *Nordic Journal of Freshwater Research* **71**, 320–323.

Kasumyan, A.O. and Sidorov, S.S. (1998) Taste preferences in young Arctic char, *Salvelinus alpinus*, brook trout, *S. fontinalis*, and lake char, *S. namaycush*. In: *Biology and Evolution of Chars of the Nothern Hemisphere*, 18 pp.

Kasumyan, A.O., Sidorov, S.S., Kazhlaev, A.A. and Pashchenko, N.I. (1995) In: *2nd International Symposium on Sturgeons* (eds A.D. Ershanovich and T.I.J. Smith). VNIRO Publishing, Moscow, pp. 278–288.

Kasumyan, A.O., Sidorov, S.S. and Pashchenko, N.I. (1993) Effect of water temperatures on taste sensitivity of fry of the stellate sturgeon *Acipenser stellatus* to free amino acids. *Doklady Biological Sciences* **331**, 265–267.

Kasumyan, A.O., Sidorov, S.S., Pashchenko, N.I. and Nemchinov, A.V. (1992) Extra- and intraoral sensitivity to amino acids in young sturgeon, *Acipenser gueldenstaedti*. *Doklady Biological Sciences* **322**, 82–84.

Kasumyan, A.O. and Taufik, L.R. (1994) Behavioral reaction of juvenile sturgeons (Acipenseridae) to amino acids. *Journal of Ichthyology* **34**, 90–103.

Kats, L.B., Petranka, J.W. and Sih, A. (1988) Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* **69**, 1865–1870.

Kawamura, G. and Washiyama, N. (1989) Ontogenetic changes in behavior and sense organ morphogenesis in largemouth bass and *Tilapia nilotica*. *Transactions of the American Fisheries Society* **118**, 203–213.

Kerfoot, W.C., Kellog, D.L. Jr and Strickler, J.R. (1980) Visual observations of live zooplankters: evasion, escape, and chemical defenses. In: *Evolution and Ecology of Zooplnakton Communities*. (ed. W.C. Kerfoot). University Press of New England, New Hampshire, pp. 0–27.

Kerr, J.N.Q. and Paul, V.J. (1995) Animal–plant defense association: the soft coral *Sinularia sp.* (Chidaria, Alcyonacea) protects *Halimeda spp.* from herbivory. *Journal of Experimental Marine Biology and Ecology* **186**, 183–205.

Kinnamon, J.C. (1987) Organization and innervation of taste buds. In: *Neurobiology of Taste and Smell*. (eds T.E. Finger and W.L. Silver). Wiley, New York, pp. 277–297.

Kinnel, R.C., Dieter, R.K., Meinwald, J. *et al.* (1979) Brasilenyne and cys-dihydrorhopophytin: antofeedant medium-ring haloethers from a sea-hare (*Aplysia brasiliana*). *Proceedings of the National Academy of Science USA* **76**, 3576–3579.

Kiyohara, S., Yamashita, S. and Harada, S. (1981) High sensitivity of minnow gustatory receptors to amino acids. *Physiology and Behavior* **26**, 1103–1108.

Klaprat, D.A., Evans, R.E. and Hara, T.J. (1992) Environmental contaminants and chemoreception in fishes. In: *Fish Chemoreception*. (ed. T.J. Hara). Chapman & Hall, London, pp. 321–341.

Knights, B. (1985) Feeding behaviour and fish culture. In: *Nutrition and Feeding in Fish*. (eds C.B. Cowey, A.M. Mackie and J.G. Bell). Academic Press, London, pp. 223–241.

Komak, S. and Crossland, M.R. (2000) An assessment of the introduced mosquito fish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildlife Research* **27**, 185–189.

Konishi, J., Hidaka, I., Toyota, M. and Matsuda, H. (1969) High sensitivity of the palatal chemoreceptors of carp to carbon dioxide. *Japanese Journal of Physiology* **19**, 327–341.

Konishi, J. and Zotterman, Y. (1961) Taste functions in the carp: an electrophysiological study on gustatory fibres. *Acta Physiologica Scandinavica* **52**, 150–161.

Konosu, S., Cheng, Y.-N. and Hashimoto, Y. (1966) Constituents of the extracts of a marine worm, *Perinereis brevicirrus*. *Bulletin of the Japanese Society of Scientific Fisheries* **32**, 881–886.

Konosu, S., Fujimoto, K., Takashima, Y., Matsushita, T. and Hashimoto, Y. (1965) Constituents of the extracts and amino acids composition of the protein of short-necked clam. *Bulletin of the Japanese Society of Scientific Fisheries* **31**, 680–686.

Konosu, S. and Hayashi, T. (1975) Determination of β-alanine betaine and glycine betaine in some marine invertebrates. *Bulletin of the Japanese Society of Scientific Fisheries* **41**, 743–746.

Kotrschal, K. (1991) Solitary chemosensory cells – taste, common chemical sense or what? *Review in Fish Biology and Fisheries* **1**, 3–22.

Kotrschal, K. (1992) Quantitative electron microscopy of solitary chemoreceptor cells in cyprinids and other teleosts. *Environmental Biology of Fishes* **35**, 273–282.

Kotrschal, K. (1995) Ecomorphology of solitary chemosensory cell systems in fish: a review. *Environmental Biology of Fishes* **44**, 143–155.

Kotrschal, K. (1996) Solitary chemosensory cells: why do primary aquatic vertebrates need another taste system? *Trends in Ecology and Evolution* **11**, 110–114.

Kotrschal, K. (2000) Taste(s) and olfaction(s) in fish: a review of spezialized sub-systems and central integration. *Pflüger Archiv – European Journal of Physiology* **439** (Suppl.), R178–R180.

Kotrschal, K. and Finger, T.E. (1996) Secondary connection of the dorsal and ventral facial lobes in a teleost fish, the rockling (*Ciliata mustela*). *Journal of Comparative Neurology* **370**, 415–426.

Kotrschal, K., Van Staaden, M.J. and Huber, R. (1998) Fish brains: evolution and environmental relationships. *Review in Fish Biology and Fisheries* **8**, 1–36.

Kotrschal, K., Whitear, M. and Finger, T.E. (1993) Spinal and facial innervation of the skin in the gadid fish, *Ciliata mustela* (Teleostei). *Journal of Comparative Neurology* **331**, 407–417.

Krinner, M. (1934) Uber die Geschmacksempfindlichkeit der Ellritzen. *Zeitschrift für Vergleichende Physiologie* **21**, 317–242.

Kruse, K.C. and Francis, M.G. (1977) A predation deterrent in larvae of the bullfrog, *Rana catesbeiana*. *Transections of the American Fisheries Society* **106**, 248–252.

Kruzhalov, N.B. (1986) Behavioral responses of carp fingerlings *Cyprinus carpio* L. to amino acids. *Voprosii Ikhtiologii* **26**, 1016–1022 (in Russian).

Kubitza, F., Lovshin, L.L. and Lovell, R.T. (1997) Identification of feed enhancers for juvenile largemouth bass *Micropterus salmoides*. *Aquaculture* **148**, 191–200.

La Barre, S.C., Coll, J.C. and Sammarco, P.W. (1986) Competitive strategies of soft corals (Coelenterata: Octocorallia). Part III. Spacing and agressive interactions between alcyonaceans. *Marine Ecology Progress in Series* **28**, 147–156.

 © 2003 Blackwell Publishing Ltd,

Lamb, C. and Finger, T.E. (1995) Gustatory control of feeding behavior in goldfish. *Physiology and Behaviour* **57**, 483–488.

Larher, T., Hamelin, J. and Stewart, G.R. (1977) L'acide dimethylsulfonium-3 propanoque de *Spartina anglica*. *Phytochemistry* **16**, 2019–2020.

Lemm, C.A. (1983) Growth and survival of Atlantic salmon fry on semimoist or dry starter diets. *Progressive Fish-Culturist* **45**, 72–74.

Leydig, F. (1851) Über die aussere Haut einiger Süsswasserfische. *Zeitschrift für Wissenschaftliche Zoologie* **3**, 1–12.

Liem, K.F. (1973) Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Systematic Zoology* **22**, 425–441.

Lindstedt, K.J. (1971) Chemical control of feeding behaviour. *Comparative Biochemistry and Physiology* **39A**, 553–581.

Lokensgard, J., Smith, R.L., Eisner, T. and Meinwald, J. (1993) Pregnanes from defensive glands of a belostomatid bug. *Experientia* **49**, 175–176.

Lucas, J.S., Hart, R.J., Howden, M.E. and Salathe, R. (1979) Saponins in eggs and larvae of *Acanthaster planci* (L.) (Asterioidea) as chemical defenses against planktivorous fish. *Journal of Experimental Marine Biology and Ecology* **49**, 155–165.

Mackie, A.M. (1982) Identification of the gustatory feeding stimulants. In: *Chemoreception in Fishes*. (ed. T.J. Hara). Elsevier Scientific Publishing Co., Amsterdam, pp. 275–291.

Mackie, A.M. (1987) Prelimenary studies on the chemical defenses of the British octocorals, *Alcyonium digitatum*, and *Pennatula phosphorea*. *Comparative Biochemistry and Physiology* **86A**, 629–632.

Mackie, A.M. and Adron, J.W. (1978) Identification of inosine and inosine-5′-monophosphate as the gustatory feeding stimulants for the turbot, *Scophthalmus maximus*. *Comparative Biochemistry and Physiology* **60A**, 79–83.

Mackie, A.M., Adron, J.W. and Grant, P.T. (1980) Chemical nature of feeding stimulants for the juvenile Dover sole, *Solea solea* (L.). *Journal of Fish Biology* **16**, 701–708.

Mackie, A.M. and Mitchell, A.I. (1982a) Chemical ecology and chemoreception in the marine environment. *Publications Du Centre national pour l'exploitation des océans, Actes de colloques* **14**, 11–24.

Mackie, A.M. and Mitchell, A.I. (1982b) Further studies on the chemical control of feeding behaviour in the Dover sole, *Solea solea*. *Comparative Biochemistry and Physiology* **73A**, 89–93.

Mackie, A.M. and Mitchell, A.I. (1983) Studies on the chemical nature of feeding stimulants for the juvenile European eel, *Anguilla anguilla* (L). *Journal of Fish Biology* **22**, 425–430.

Mackie, A.M. and Mitchell, A.I. (1985) Identification of gustatory feeding stimulants for fish applications in aquaculture. In: *Nutrition and Feeding in Fish*. (eds C.B. Cowey, A.M. Mackie and J.G. Bell). Academic Press, London, pp. 177–189.

Marui, T. and Caprio, J. (1992) Teleost gustation. In: *Fish Chemoreception*. (ed. T.J. Hara). Chapman & Hall, London, pp. 171–198.

Marui, T., Caprio, J., Kiyohara, S. and Kasahara, Y. (1988) Topographical organization of taste and tactile neurons in the facial lobe of the sea catfish, *Plotosus lineatus*. *Brain Research* **446**, 178–182.

Marui, T., Evans, R.E., Zielinski, B. and Hara, T.J. (1983a) Gustatory responses of the rainbow trout (*Salmo gairdneri*) palate to amino acids and derivates. *Journal of Comparative Physiology* **153A**, 423–433.

Marui, T., Harada, S. and Kasahara, Y. (1983b) Gustatory specifity for amino acids in the facial taste system of the carp, *Cyprinus carpio* L. *Journal of Comparative Physiology* **153A**, 299–308.

Marui, T. and Kiyohara, S. (1987) Structure-activity relationships and response features for amino acids in fish taste. *Chemical Senses* **12**, 265–275.

McBride, J.R., Idler, D.R., Jones, R.E. and Tomlinson, N. (1962) Olfactory perception in juvenile salmon. Part I. Observation on response of juvenile sockeye to extracts of food. *Journal of the Fisheries Research Board of Canada* **19**, 327–334.

McClintock, J.B. and Janssen, J. (1990) Pteropod abduction as a chemical defense in a pelagic Antarctic amphipod. *Nature* **346**, 462–464.

McClintock, J.B., Swenson, D.P., Steinberg, D.K. and Michaels, A.A. (1996) Feeding-deterrent properties of common oceanic holoplankton from Bermudian waters. *Limnology and Oceanography* **41**, 798–801.

Mearns, K.J. (1986) Sensitivity of brown trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.) fry to amino acids at the start of exogenous feeding. *Aquaculture* **55**, 191–200.

Mearns, K.J., Ellingsen, O.F., Døving, K.B. and Helmer, S. (1987) Feeding behaviour in adult rainbow trout and atlantic salmon parr, elicited by chemical fractions and mixtures of compounds identifited in shrimp extract. *Aquaculture* **64**, 47–63.

Metcalfe, N.B., Huntingford, F.A. and Thorpe, J.E. (1986) Seasonal changes in feeding motivation of juvenile Atlantic salmon (*Salmo salar*). *Canadian Journal of Zoology* **64**, 2439–2446.

Meyer, A. (1988) Influence of age and size on the response to novel prey by fish of the cichlid fish *Cichlasoma managuense* (Pisces: Cichlidae). *Ethology* **78**, 199–208.

Meyer, K.D. and Paul, V.J. (1995) Variation in secondary metabolite and aragonite concentrations in the tropical green seaweed *Neomeris annulata*: effects on herbivory by fishes. *Marine Biology* **122**, 537–545.

Michel, W. and Caprio, J. (1991) Responses of single facial taste fibers in the sea catfish, *Arius felis*, to amino acids. *Journal of Neurophysiology* **66**, 247–260.

Millkin, M.R. (1982) Qualitative and quantitative nutrient requirements of fishes: a review. *Fisheries Bulletin* **80**, 655–685.

Mistretta, C.M. (1991) Developmental neurobiology of the taste system. In: *Smell and Taste in Health and Disease*. (eds T.V. Getchell, L.M. Bartoshuk, R.L. Doty and J.B. Snow). Raven Press, New-York, pp. 35–64.

Miyagawa, M., Nakamoto, S., Yamane, K. *et al.* (1979) Studies on organic constituents of the snow crab (*Chionoecetes opillio*). Part II. Free amino acids in extracts. *Bulletin of the Japanese Society of Scientific Fisheries* **45**, 115–120.

Morita, Y. and Finger, T.E. (1985) Topographic and laminar organization of the vagal gustatory system in the goldfish, *Carassius auratus*. *Journal of Comparative Neurology* **238**, 187–201.

Motta, P.J. (1982) Functional morphology of the head of the intertial suction feeding butterfly fish, *Chaetodon miliaris* (Perciformes, Chaetodontidae). *Journal of Morphology* **174**, 283–312.

Motta, P.J. (1988) Functional morphology of the feeding apparatus of ten species of Pacific butterfly fishes (Perciformes, Chaetodontidae): an ecomorphological apporoach. *Environmental Biology of Fishes* **22**, 39–67.

Murofushi, S. and Ina, K. (1981) Survey of feeding stimulants for the sea bream present in the dried pupae of silkworms. *Agricultural and Biological Chemistry* **45**, 1501–1504.

Nakajima, K. (1991) Effects of diet-supplemented dimethyl-β-propiothetin on growth and trust power of goldfish, carp and red sea bream. *Nippon Suisan Gakkashi* **57**, 673–679.

Nakajima, K., Uchida, A. and Ishida, Y. (1989a) Effect of supplemental dietary feeding attractant, dimethyl-β-propiothetin, on growth of goldfish. *Nippon Suisan Gakkashi* **55**, 1291.

Nakajima, K., Uchida, A. and Ishida, Y. (1989b) A new feeding attractant, dimethyl-β-propiothetin, for freshwater fish. *Nippon Suisan Gakkashi* **55**, 689–695.

Nakajima, K., Uchida, A. and Ishida, Y. (1990) Effect of a feeding attractant, dimethyl-β-propiothetin, on growth of marine fish. *Nippon Suisan Gakkashi* **56**, 1151–1154.

Nakamura, M. and Kurihara, K. (1991) Differential temperature dependence of taste nerve responses to various taste stimuli in dogs and rats. *American Journal of Physiology* **261**, R1402–R1408.

Nikolaeva, E.V. and Kasumyan, A.O. (2000) Comparative analysis of the taste preferences and behavioral responses to gastatory stimuli in females and males of the guppy, *Poecilia reticulata*. *Journal of Ichthyology* **40**, 479–484.

Nikolski, G.V. (1963) *The Ecology of Fishes*. Academic Press, London.

Ohsugi, T., Hidaka, I. and Ikeda, M. (1978) Taste receptor stimulation and feeding behaviour in the puffer, *Fugu pardallis*. Part II. Effects produced by mixtures of constituents of clam extract. *Chemical Senses and Flavor* **3**, 355–368.

Oikawa, C.K. and March, B.E. (1997) A method for assessment of the efficacy of feed attractants for fish. *Progressive Fish-Culturist* **59**, 213–217.

Osinov, A.G. and Bernache, L. (1996) 'Atlantic' and 'Danube' phylogenetic groupings of brown trout, *Salmo trutta*, complex: genetic divergency, evolution and conservation. *Journal of Ichthyology* **36**, 762–786.

Osse, J.W.M., Sibbing, F.A. and Van Den Boogaart, J.G.M. (1997) Intra-oral food manipulation of carp and other cyprinids: adaptationa and limitations. *Acta Physiologica Scandinavica* **161**, 47–57.

Papatryphon, E. and Soares, J.H.J. (2000) The effect of dietary feeding stimulants on growth performance of striped bass, *Morone saxatilis*, fed a plant feed stuff-based diet. *Aquaculture* **185**, 329–338.

Paul, V.J. (1992) Chemical defenses of benthic marine invertebrates. In: *Ecological Roles of Marine Natural Products*. (ed. V.J. Paul). Comstock, Ithaca, New York, pp. 164–188.

Paul, V.J., Cronan, J.M. Jr and Cardellina, J.H.I. (1993) Isolation of new brominated sequiterpene feeding deterrents from tropical green alga, *Neomeris annulata* (Dasycladaceae: Chlorophyta). *Journal of Chemical Ecology* **19**, 1847–1860.

Pausan, K. (1984) *Aquarium Fishes*. Country Life Books, Middlesex.

Pavlov, D.S. and Kasumyan, A.O. (1990) Sensory principles of the feeding behaviour of fishes. *Journal of Ichthyology* **30**, 77–93.

Pavlov, D.S. and Kasumyan, A.O. (1998) The structure of the feeding behaviour of fishes. *Journal of Ichthyology* **38**, 116–128.

Pawlik, J.R. (1993) Marine invertebrate chemical defenses. *Chemical Review* **93**, 1911–1922.

Pawlik, J.R., Chanas, B., Toonen, R.J. and Fenical, W. (1995) Defenses of Carribbean sponges against predatory reef fish. Part I. Chemical deterrency. *Marine Ecology Progress Series* **127**, 183–194.

Pawson, M.G. (1977) The responses of cod, *Gadus morhua* (L), to chemical attractants in moving water. *Journal du conseil International pour l'exploration de la mer* **37**, 316–318.

Pevzner, R.A. (1981) Ultrastructure of taste receptors in Chondrichthyes. Part I. Adult sturgeon. *Tsitologija* **23**, 760–766 (in Russian).

Pevzner, R.A. (1985) Ultrastructure of taste receptors in Chondrichthyes. Part III. Sturgeon larvae in yolk-feeding period. *Tsitologija* **27**, 1240–1246 (in Russian).

Poe, W.E. and Wilson, R.P. (1989) Palatability of diets containing sulfadimetoxine, ormetoprim, and Romet 30 to channel vatfish fingerlings. *Progressive Fish-Culturist* **51**, 226–228.

Prescott, J. and Stevenson, R.J. (1995) Effects of oral chemical irritation on tastes and flavors in frequent and infrequent users of Chilli. *Physiology and Behavior* **58**, 117–1127.

Prezant, R.S. (1980) An antipredator mechanism of the polychaete, *Phyllodoce mucose*, with notes on similar mechanisms in other potential prey. *US Fisheries Bulletin* **77**, 605–616.

Raderman-Little, R. (1979) The effect of temperature on the turnover of taste bud cells in catfish. *Cell and Tissue Kinetics* **12**, 269–280.

Reutter, K. (1986) Chemoreceptors. In: *Biology of the Integument. Vertebrates* **Vol. 2** (eds J. Bereiter-Hahn, A.G. Matoltsy and K.S. Richards). Springer-Verlag, Berlin, pp. 586–604.

Reutter, K. (1987) Specialized receptor villi and basal cells within the taste bud of the European silurid fish, *Silurus glanis* (Teleostei). In: *Olfaction and Taste. Part IX*. (eds D. Roper and J. Atema). Annals NY Acad. Sci, NY, pp. 570–573.

Reutter, K. (1992) Structure of the peripheral gustatory organ, represented by the siluroid fish *Plotosus lineatus* (Thunberg). In: *Fish Chemoreception*. (ed. T.J. Hara). Chapman & Hall, London, pp. 60–78.

Reutter, K., Breipohl, W. and Bijvank, G.J. (1974) Taste bud types in fishes. Part II. Scanning electron microscopical investigations on *Xiphophorus helleri* Heckel (Poeciliidae, Cyprinodontiformes, Teleostei). *Cell and Tissue Research* **153**, 151–165.

Reutter, K. and Witt, M. (1993) Morphology of vertebrate taste organs and their nerve supply. In: *Mechanisms of Taste Transduction* (eds S.A. Simon and S.D. Roper). CRC Press, Boca Raton, pp. 29–82.

Rideout, J.A., Smith, N.B. and Sutherland, M.D. (1979) Chemical defense of crinoids by polyketide sulphates. *Experientia* **35**, 1273–1274.

Ringler, N.H. (1985) Individual and temporal variation in prey switching by brown trout *Salmo trutta*. *Copeia* **4**, 918–926.

Robinson, E.H., Brent, J.R., Crabtree, J.T. and Tucker, C.S. (1990) Improved palatability of channel catfish feeds containing Romet-30®. *Journal of Aquatic Animal Health* **2**, 43–48.

Robinson, E.H. and Tucker, C.S. (1992) Palatability of sarafloxin HCl-medicated feed to channel catfish. *Journal of Applied Aquaculture* **1**, 81–87.

Rogers, S.D. and Paul, V.J. (1991) Chemical defenses of three *Glossodoris nudibranchs* and their dietary Hyrtyos sponges. *Marine Ecology Progress Series* **77**, 221–232.

Saglio, P. and Blanc, J.M. (1983) Etude en olfactometre de la reponse comportementale aux L-acides amines chez le juvenile de carpe, *Cyprinus carpio* L. *Biology of Behaviour* **8**, 359–370.

Saito, T., Noguchi, T., Harada, T., Murata, O. and Hashimoto, K. (1984) Tetrodoxin as a biological defence agent for puffers. *Bulletin of the Japanese Society of Scientific Fisheries* **51**, 1175–1180.

Sakata, K. (1989) Feeding attractants and stimulants for marine gastropods. *Bioorganic Marine Chemistry* **3**, 115–129.

Sammarco, P.W. and Coll, J.C. (1990) Lack of predictability in terpenoid function: multiple roles and integration with related adaptations in soft corals. *Journal of Chemical Ecology* **16**, 273–289.

Sammarco, P.W., La Barre, C.L. and Coll, J.C. (1987) Defensive strategies of soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef. Part III. The relationship between ichthyotoxicity and morphology. *Oecologia (Berlin)* **74**, 93–101.

Saxena, S.C. (1959) Adhesive apparatus of a hill-stream cyprinid fish, *Garra mullya* (Sykes). *Proceedings of the National Institute of Sciences of India* **B25**, 205–214.

Scharrer, E., Smith, S.W. and Palay, S.L. (1947) Chemical sense and taste in the fishes, Prionotus and Trichogaster. *Journal of Comparative Neurology* **68**, 183–198.

Schildknecht, H. (1971) Evolutionary peaks in the defensive chemistry of insects. *Endeavour* **30**, 136–141.

Schreck, J.A. and Moffitt, C.M. (1987) Palatability of feed containing different concentrations of erythromycin thiocyanate to chinook salmon. *Progressive Fish-Culturist* **49**, 241–247.

Schulte, B.A. and Bakus, G.J. (1992) Predation deterrance in marine sponges: laboratory versus field studies. *Bulletin of Marine Science* **50**, 205–211.

Sheldon, R.E. (1909) The reaction of dogfish to chemical stimuli. *Journal of Comparative Neurology* **19**, 273–311.

Sibbing, F.A. (1988) Specializations and limitations in the utilization of food resources by the carp, *Cyprinus carpio*: a study of oral food processing. *Environmental Biology of Fishes* **22**, 161–178.

Sibbing, F.A. (1991) Food capture and oral processsing. In: *Cyprinid Fishes: Systematics, Biology and Exploitation* (eds I.J. Winfield and J.S. Nelson). Chapman & Hall, London, pp. 377–412.

Sibbing, F.A., Osse, J.W.M. and Terlouw, A. (1986) Food handling in the carp (*Cyprinus carpio*): its movement patterns, mechanisms and limitations. *Journal of Zoology (London)* **210A**, 161–203.

Silver, W.L. (1987) The common chemical sense. In: *Neurobiology of Taste and Smell. The Common Chemical Sense* (eds T.E. Finger and W.L. Silver). Wiley, New York, pp. 65–87.

Silver, W.L. and Finger, T.E. (1991) The trigeminal system. In: *Smell and Taste in Health and Desease*. (eds T.V. Getchell, L.M. Bartoshuk, R.L. Doty and J.B. Snow). Raven Press, New York. pp. 97–108.

Specziar, A., Tolg, L. and Biro, P. (1997) Feeding strategy and growth of cyprinids in the littoral zone of Lake Balaton. *Journal of Fish Biology* **51**, 1109–1124.

Stevens, D.A. (1990) Personality variables in the perception of oral irritation and flavor. In: *Chemical Senses, Irritation*, **Vol. 2** (ed. B.G. Green) edn New York, pp. 217–228.

Storebakken, T. and Austreng, E. (1988) Feed intake measurements in fish using radioactive isotopes. Part II. Experiments with Atlantic salmon and rainbow trout in sea-pens. *Aquaculture* **70**, 277–288.

Storebakken, T., Austreng, E. and Steenberg, K. (1981) A method for determination of feed intake in salmonids using radioactive isotopes. *Aquaculture* **24**, 133–142.

Stradmeyer, L. (1989) A behavioural method to test feeding responses of fish to pelleted diets. *Aquaculture* **79**, 303–310.

Stradmeyer, L., Metcalfe, N.B. and Thorpe, J.E. (1988) Effect of food pellet shape and texture on the feeding response of juvenile Atlantic salmon. *Aquaculture* **73**, 217–228.

Stradmeyer, L. and Thorpe, J.E. (1987) The responses of hatcheryreared Atlantic salmon, *Salmo salar* L. parr to pelletted and wild prey. *Aquaculture and Fisheries Management* **18**, 51–61.

Strieck, F. (1924) Untersuchungen uber den Geruchs- und Geschmackssinn der Elritze (*Phoxinus laevis*). *Zeitschrift fürVergleichende Physiologie* **2**, 122–154.

Stuart, J. (1996) *How to Keep Tropical Aquarium Fish.* Tiger Books Intern, London.

Sutterlin, A.M. (1975) Chemical attraction of some marine fish in their natural habitat. *Journal of the Fisheries Research Board of Canada* **32**, 729–738.

Sutterlin, A.M. and Sutterlin, N. (1970) Taste responses in Atlantic salmon (*Salmo salar*) parr. *Journal of the Fisheries Research Board of Canada* **27**, 1927–1942.

Takaoka, O., Takii, K., Nakamura, M., Kumai, H. and Takeda, M. (1990) Identification of feeding stimulants for marbled rockfish. *Bulletin of the Japanese Society of Scientific Fisheries* **56**, 345–351.

Takeda, M. and Takii, K. (1992) Gustation and nutrition in fishes: application to aquaculture. In: *Fish Chemoreception* (ed. T.J. Hara). Chapman & Hall, London, pp. 271–287.

Takeda, M., Takii, K. and Matsui, K. (1984) Identification of feeding stimulants for juvenile eel. *Bulletin of the Japanese Society of Scientific Fisheries* **50**, 645–651.

Takii, K., Shimeno, S., Takeda, M. and Kamekawa, S. (1986) The effect of feeding stimulants in diet on digestive enzyme activities of eel. *Bulletin of the Japanese Society of Scientific Fisheries* **52**, 1449–1454.

Takii, K., Takeda, M. and Nakao, Y. (1984) Effect of supplement of feeding stimulants to formulated feeds on feeding activity and growth of juvenile eel. *Bulletin of the Japanese Society of Scientific Fisheries* **50**, 1039–1043.

Talbot, C. (1985) Laboratory methods in fish feeding and nutritional studies. In: *Fish Energetics: New Perspectives* (eds P. Tytler and P. Calow). Croom-Helm, London, pp. 125–154.

Talbot, C. and Higgins, P.J. (1983) A radiographic method for feeding studies on fish using metallic iron powder as a marker. *Journal of Fish Biology* **23**, 211–220.

Tandler, A., Berg, B.A., Kissil, G.W. and Mackie, A.M. (1982) Effect of food attractants on appetite and growth rate of gilthead bream, *Sparus aurata L. Journal of Fish Biology* **20**, 673–681.

Tester, A.L. (1963) Olfaction, gustation and the common chemical sense in sharks. In: *Sharks and Survival.* (ed. P.W. Gilbert). D.C. Heath, Boston, pp. 255–282.

Thacker, R.W., Nagle, D.G. and Paul, V.J. (1997) Effects of repeated exposure to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. *Marine Ecology Progress Series* **147**, 21–29.

Thompson, J.E., Walker, R.P. and Faulkner, D.J. (1985) Screening and bioassays for biologically-active substances from forty marine sponge species from SanDiego, California, USA. *Marine Biology* **88**, 11–21.

Toften, H., Jorgensen, E.H. and Jobling, M. (1995) The study of feeding preferences using radiography: oxytetracycline as a feeding deterrent and squid extract as a stimulant in diets for Atlantic salmon. *Aquaculture Nutrition* **1**, 145–149.

Trudel, P.J. (1929) Untersuchungen über Geschmacksreaktion der Fische auf 'süsse' Stoffe. *Zeitschrift für Vergleichende Physiologie* **10**, 367–409.

Tucker, D. (1983) Fish chemoreception: periferal anatomy and physiology. In: *Fish Neurobiology: Brainstem and Sensory Organs*, **Vol. 1** (eds R.G. Northcutt and R.E. Davis). University of Michgan Press, Ann Arbor, pp. 311–349.

von Uexküll, J. (1895) Vergleichend-sinnesphysiologische Untersuchungen. I. Über die Nahrungsaufnahme des Katzenhais. *Zeitschrift für Biologie* **32**, 548–566.

Van Damme, R., Bauwens, D., Vanderstighelen, D. and Verheyen, R.F. (1990) Responses of the lizard *Lacerta vivipara* to predator chemical cues: the effects of temperature. *Animal Behaviour* **40**, 298–305.

Van Diggelin, J., Rozema, J., Dickson, D.M.J. and Broekman, R. (1986) β-3-Dimethylsulphoniopropionate, proline and quaternary ammonium compounds in *Spartice anglica* in relation to sodium chloride, nitrogen and sulphur. *New Phytologist* **103**, 573–586.

Vasil'ev, V.P. (1985) *Evolutionary Karyology of Fish.* Nauka, Moscow.

Voris, H.K. and Bacon, J.P. (1966) Differential predation on tadpoles. *Copeia* **3**, 594–598.

Weber, E.H. (1827) Über das Geschmacksorgane des Karpfen and den Ursprung seiner Nerven. *Archiv für Anatomie and Physiologie*, 309–315.

Welsch, U. and Storch, V. (1969) Die Feinstruktur der Geschmacksknospen von Welsen [*Clarias batrachus* (L.) and *Kryptopterus bicirrhis* (Cuvier et Valenciennes)]. *Zeitschrift für Zellforschung und Mikroscopische Anatomie* **100**, 552–559.

Whitear, M. (1971) Cell specialization and sensory function in fish epidermis. *Journal of Zoology (London)* **163**, 237–264.

Whitear, M. (1992) Solitary chemosensory cells. In: *Fish Chemoreception* (ed. T.J. Hara). Chapman & Hall, London, pp. 103–125.

Whitear, M. and Kotrschal, K. (1988) The chemosensory anterior dorsal fin in rockling (Gaidropsarus and Ciliata, Teleostei, Gadidae): activity, fine structure and innervation. *Journal of Zoology (London)* **216**, 339–366.

Whitear, M. and Moate, R.M. (1994) Chemosensory cells in the oral epithelium of *Raja clavata* (Chondrichthyes). *Journal of Zoology (London)* **232**, 295–312.

Wilson, D.M., Puyana, M., Fenical, W. and Pawlik, J.R. (1999) Chemical defense of the Caribbean reef sponge *Axilella*

 © 2003 Blackwell Publishing Ltd,

corrugata against predatory fishes. *Journal of Chemical Ecology* **25**, 2811–2824.

Winn, H.E. and Bardach, J.E. (1959) Differential food selection by moray eels and a possible role of the mucous envelope of parrot fishes in reduction of predation. *Ecology* **40**, 296–298.

Witt, M. and Reutter, K. (1990) Electron microscopic demonstration of lectin binding sites in the taste buds of the European catfish *Silurus glanis* (Teleostei). *Histochemistry* **94**, 617–628.

Wootton, R.J. (1998) *Ecology of Teleost Fishes.* Kluwer Academic Publishers, Dordrecht.

Wylie, C.R. and Paul, V.J. (1988) Feeding preference of the surgeon fish *Zebrasoma flavescens* in relation to chemical defenses of tropical algae. *Marine Ecology Progress Series* **45**, 23–32.

Yamada, S. and Yone, Y. (1986) Loss of dietary amino acids during mastication by carp. *Bulletin of the Japanese Society of Scientific Fisheries* **52**, 673–676.

Yamamori, K., Nakamura, M., Matsui, T. and Hara, T.J. (1988) Gustatory responses to tetrodoxin and saxitoxin in fish: a possible mechanism for avoiding marine toxins. *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 2182–2186.

Yamashita, S. (1964) Chemoreceptor response in frog as modified by temperature change. *Japanese Journal of Physiology* **14**, 488–504.

Yoshii, K., Kamo, N., Kurihara, K. and Kobatake, Y. (1979) Gustatory responses of eel palatine receptors to amino acids and carboxylic acids. *Journal of General Physiology* **74**, 301–317.

Yoshii, K., Kashiwayanagi, M., Kurihara, K. and Kabataki, Y. (1980) High sensitivity of eel palatine receptors to carbon dioxide. *Comparative Biochemistry and Physiology* **66A**, 327–330.

Yoshiyama, R.M. and Darling, J.D.S. (1982) Grazing by the intertidal fish *Anoplarchus purpurescens* upon a distasteful polychate worm. *Environmental Biology of Fishes* **7**, 39–45.

Zimmer-Faust, R.K. (1987) Crustacean chemical perception: towards a theory on optimal chemoreception. *Biological Bulletin* **172**, 10–29.