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REVIEW ARTICLE

Neurohormonal regulation of feed intake and response to nutrients in fish: aspects of feeding rhythm and stress

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Abstract

The regulation of feed intake is very complex and involves interaction among the circadian and homeostatic control systems within the central nervous system, the gastrointestinal tract and the environment. The hypothalamus, which receives, integrates and transmits relevant internal and external signals, is recognized as the primary centre of regulation of feed intake. The neuroendocrine factors that originate from the hypothalamus either stimulate or inhibit feed intake so that nutritional demands of the organism can be fulfilled and energy balance can be achieved. Appetite regulation is a physiological mechanism in which a variety of neurohormones interact and fish show different feeding behaviour (e.g. diurnal, nocturnal). This complicated system is very sensitive to any disturbance. Fish in farms and fish in a natural environment are equipped with the same combination of neurohormones to regulate feed intake, but they meet different challenges, particularly with regard to the type of feed and feeding schedule. In this review, the neurohormonal regulation of feed intake is analysed in fish in terms of entrainment of their circadian feeding rhythms and while exposed to different stressors in captivity.

Keywords: neurohormones, brain mechanisms, appetite, feeding behaviour, circadian rhythms, welfare

Introduction

The regulation of feeding requires interaction among the central nervous system (CNS), the gastrointestinal tract (GIT) and the environment. In vertebrates, including fish, the hypothalamus is the primary centre of regulation of feed intake. The hypothalamus receives, integrates and transmits relevant internal and external signals (Naslund & Hellstrom 2007). The neuroendocrine factors that originate from the hypothalamus either stimulate or inhibit feed intake in fish. They have been reviewed at length in many papers (Lin, Volkoff, Narnaware, Bernier, Peyon & Peter 2000; Volkoff, Canosa, Unniappan, Cerda-Reverter, Bernier, Kelly & Peter 2005; Gorissen, Flik & Huising 2006; Volkoff 2006; Volkoff & Peter 2006) and, therefore, here they are only briefly presented. There are still many issues concerning the circadian and neurohormonal regulation of feeding, which should be discussed, and this paper deals with some of them, specifically relevant to intensive fish farming in captivity.

It is clear that most fish do not feed continuously during 24 h, but display particular day/night rhythmic patterns. Although a few species that consume low-energy food, such as grass carp (*Ctenopharyngodon idella*), may need to forage almost continuously to obtain the energy they need, distinct feeding rhythms are widespread in fish. Such temporal organization of feeding behaviour has evolved under the influence of cyclic selective forces. Indeed, most physiological and behavioural functions show rhythms directed by a biological clock, which enables animals to anticipate cyclic events in their environment and mount an appropriate response (Pittendrigh 1993). Several reviews of rhythmicity in fish reported in a number of species the existence of consistent patterns, in some cases with diurnal fish becoming nocturnal and vice versa at a certain time (Thorpe 1978; Ali 1992). At first, such dualism in the phasing of daily rhythms was believed to be species dependent and associated with fish living at high latitudes. However, soon after other species living in temperate regions were reported to exhibit flexibility in their daily rhythms. For instance, sea bass brought from sea cages to laboratory tanks and kept singly or in groups displayed either diurnal or nocturnal feeding rhythms (Sánchez-Vázquez, Madrid, & Zamora 1995). Most strikingly, the same individuals could shift their phasing, under constant laboratory conditions, without apparent changes in their environment. Dual phasing of daily feeding rhythms has also appeared in goldfish *Carassius auratus*, which mostly feed during daytime, but sometimes may change one to nighttime. However, not all fish are equipped with the dual feeding strategy, and some species show simpler feeding rhythms. Some fish, for instance, have a more rigid feeding phase constrained by their sensory capacity: visual feeders (e.g. trout) tend to be diurnal, while fish with poor vision (e.g. catfish) are mostly nocturnal. Reebs (2002) and Madrid, Boujard and Sánchez-Vázquez (2001) provide further examples of the plasticity of the fish feeding patterns.

Fish in farms and fish in a natural environment show no differences in neurohormones that regulate feed intake, but they meet different challenges. Wild fish have their own pattern of feeding: some eat almost continuously while some, especially predators, may feed intermittently with periods of enforced fasting. In fish, cold-blooded poikilotherms, the patterns of activity and metabolic requirements strongly depend on environmental temperature and are reflected in the frequency of feeding. A relationship between feeding rhythms and either temperature or photoperiod has been demonstrated in many fish species. The marked changes in appetite also depend on life-history events. For instance, juvenile salmon become naturally anorectic, eating little for weeks in the winter, while maturing salmon show a peak in appetite in spring, when nutrients for migration and spawning are accumulated (Kadri, Thorpe & Metcalfe 1997). Fish also have the capacity to select a diet related to their specific nutritional demand. Nutrient requirements of young growing fish and adult ones, during spawning and out of the breeding season, are also different. On the other hand, farmed fish are fed artificial diets and the farmer controls the timing of supply. Wild fish naturally experience a variety of adverse conditions, such as attacks of predators or conspecifics, starvation, exposure to poor water quality, etc. Similarly, fish in aquaculture are subjected to many unfavourable conditions, but they are different from that in a natural environment, for instance, crowding, which induces aggressiveness within a group, disturbing and handling, unnatural light–dark regimes. All of these strongly affect feeding behaviour.

This paper focuses on the analysis of the chronobiological and homeostatic regulation of feed intake in fish during exposure to stress and the entrainment of circadian feeding rhythms. Such an approach offers a new insight into feed intake control in fish in captivity, especially while subjected to stress and fixed scheduled feeding.

Major substances involved in the regulation of feed intake

It is established that the hypothalamus is a major site of the control of feed intake in the brain. As early as in the 1970s, electric stimulation of inferior lobes of the hypothalamus, ventral telencephalon and the optic tectum was shown to elicit a strong feeding response in several fish species (Demski & Knigge 1971; Demski 1973; Roberts & Savage 1978). It was also suggested that the input from the telencephalon and the optic tectum could activate hypothalamic centres and evoke feeding behaviour. The hypothalamus is continuously informed about the nutritional, energetic and environmental status of the organism through peripheral and central orexigenic and anorexigenic messages. The peripheral feedback signals, including nervous inputs, GIT peptides, leptin, cortisol, glucose and insulin, are integrated by the feeding centre in the hypothalamus. Neuropeptides and monoamines convey the major central signals.

In the 1990s, a number of fish appetite-regulating peptides, homologous to those in mammals, have been identified (for a review, see Lin *et al.* 2000; Volkoff *et al.* 2005; Volkoff 2006; Volkoff & Peter 2006). Most of them have been found in both the brain and the GIT. Until now, many peptides have been isolated or their amino acid sequence has been deduced from cloned cDNA sequences. These peptides include both the orexigenic and the anorexigenic factors.

Neuropeptide Y (NPY)-related peptides are the most potent central enhancers of appetite in fish. Neuropeptide Y neurones are widely distributed in the CNS and NPY-immunoreactive fibres have been identified in the pituitary and GIT of many fish species (for a review, see Volkoff *et al.* 2005; Volkoff

2006). The highest levels of NPY mRNA are present in the telencephalon-preoptic region, in the hypothalamus and the optic tectum thalamus in goldfish (Narnaware, Peyon, Lin & Peter 2000). In chinook salmon, fasted for prolonged periods of time, an increased NPY-like gene expression was found in the preoptic area (POA) of the hypothalamus (Silverstein, Breininger, Baskin & Plisetskaya 1998). Also in goldfish, food deprivation resulted in increased brain NPY mRNA levels (Narnaware *et al.* 2000). An increase in brain NPY was reported as an effect of 3 days of food deprivation in goldfish; after re-feeding this effect was reversed (Narnaware & Peter 2001). It was also shown in the same species that NPY gene expression, in specific brain areas, associated with control of feeding behaviour, could be influenced by diet composition, i.e. high-carbohydrate and high-fat contents. Thus, it has been proposed that NPY may be involved not only in the control of feed intake but also in the macronutrient choice, at least in goldfish (Narnaware & Peter 2002). However, until now, the physiological mechanism of diet-selecting capacity in fish remains unknown.

Orexigenic peptides, including orexin A and B, galanin, the agouti-related protein (AgRP) and ghrelin, have been identified in teleost fish (Volkoff 2006). Orexins are produced by cleavage of one precursor: prepro-orexin. In goldfish, orexin-immunoreactive cells were reported in the telencephalon, thalamus and hypothalamus. In zebrafish *Danio rerio*, prepro-orexin mRNA and orexin peptides were demonstrated in the hypothalamus. Moreover, also in zebrafish, brain levels of prepro-orexin mRNA increased after long-term food deprivation (Novak, Jiang, Wang, Teske, Kotz & Levine 2005). In goldfish, orexin A was shown to be more potent than orexin B in stimulation of feeding behaviour and feed intake (Volkoff, Bjorklund & Peter 1999). A large number of orexigenic peptides axons were presented in direct synaptic contact with NPY-secreted cells, thus suggesting action through the regulation of the NPY system. Actually, it has been demonstrated that most of the peptides interact with NPY in the control of feed intake, but they can also act independently. Neuropeptide Y and orexin A seem to act synergically in goldfish (Volkoff & Peter 2001). Ghrelin, for instance, has not been co-localized with other appetite-regulatory peptide mRNAs, but its mRNA is expressed in the brain region in goldfish that regulate feed intake (Unniappan & Peter 2005). The pattern of synthesis, secretion and action of ghrelin, which have been presented in this species, points to its importance as an orexigenic factor (Unniappan, Canosa & Peter 2004). Also in goldfish, preprogalanin mRNA was reported in brain sites involved in the regulation of feed intake (Unniappan, Cerdá-Reverter & Peter 2004). The changes in its mRNA expression are related to the feeding support orexigenic action of the active peptide, galanin. The orexigenic effect of galanin was shown to be mediated by α_2-adrenergic receptors (De Pedro, Cespedes, Delgado & Alonso-Bedate 1995).

Many potent anorexigenic factors are also present in fish such as cholecystokinin (CCK) – gastrin, cocaine and amphetamine-regulated transcript (CART) and corticotropin-releasing factor (CRF). Cholecystokinin and CART have synergistic effects on feed intake and can modulate the actions of NPY and orexins (Volkoff *et al.* 2005). Moreover, CCK and CART gene expression in the hypothalamus is induced by central administration of mammalian leptin in goldfish. The leptin-like protein, which was recently identified in several fish species, is probably an important anorexigenic factor, similar to leptin in mammals (De Pedro, Martinez-Alvarez & Delgado 2006). The effect of leptin on feed intake seems to be mediated, at least in part, by CCK (Volkoff 2006). In the 2000s, more anorexigenic factors, including bombesin (BBS or gastrin-releasing peptide – GRP), α-melanocyte-stimulating hormone (α-MSH), melanin-concentrating hormone (MCH), tachykinins and urotensin I (UI), have been identified in teleosts (Lin *et al.* 2000; Volkoff *et al.* 2005). In goldfish, the α-MSH signalling pathway seems to mediate an anorexigenic action of MCH in the brain. This effect is accompanied by decreased synthesis of orexigenic peptides, NPY and ghrelin (Shimakura, Miura, Maruyama, Nakamachi, Uchiyama, Kageyama, Shioda, Takahashi & Matsuda 2008). Corticotropin-releasing factor and UI, as elements of the hypothalamus–pituitary–interrenal (HPI) stress axis, can reduce appetite in fish in response to environmental, social and physical stressors (Bernier 2006).

Besides peptides, there are other signal molecules like hypothalamic monoamine neurotransmitters involved in the control of feed intake in fish. In goldfish, both norepinephrine (NE) and dopamine (DA) are shown to be involved in the hypothalamic response to starvation; the noradrenergic system is activated and the dopaminergic system is inhibited by fasting (De Pedro, Delgado & Alonso-Bedate 2001). In goldfish, feeding is centrally stimulated by NE via α_2-adrenergic receptors and reduced after stimulation of α_1-adrenergic, and D_1 and D_2 dopaminergic receptors (De Pedro, Cespedes, *et al.* 1995; De Pedro, Delgado, Pinillos & Alonso-Bedate 1998). Serotonin (5-hydroxytryptamine; 5-HT) is also involved in the control of feeding behaviour in fish, acting as an anorectic. In rainbow trout *Oncorhynchus mykiss*, administration of the 5-HT-releasing agent, fenfluramine, inhibits feed intake (Ruibal, Soengas & Aldegunde 2002). Serotonin central anorectic action may be independent or mediated, at least in part, by CRF, as was shown in goldfish (De Pedro, Pinillos, Valenciano, Alonso-Bedate & Delgado 1998).

Moreover, the role of sensory inputs (oral factors) on feed intake control should be considered. Several mechanisms, involving both learning and physiological processes, have been proposed. With regard to learning processes, it has been argued that once a food item is consumed, animals learn to associate its orosensory properties (taste, smell and texture) with its post-ingestive consequences. For example, if an animal ingests a toxic food, it will develop a conditioned aversion to its flavour, whereas if the post-ingestive effects are positive, the animal will acquire a preference for its flavour (Sclafani 2000). Such flavour–nutrient associations appear to be learned through individual experience influenced by parental and other social interactions. Regarding physiological mechanisms, some authors have proposed different innate processes to explain specific appetites for macronutrients, mostly in mammals. Rats appear to have an innate preference for the taste of some nutrients such as sugar, maltodextrins and some types of starch, fats and proteins (Deutsch, Moore & Heinrichs 1989). In addition, there are examples of neurotransmitters, such as serotonin, DA, NPY and galanin, which differentially affect the consumption of fats and carbohydrates (Leibowitz 2000).

The mechanisms that have been proposed for food discrimination, whether through learning or innate processes, assume the oropharyngeal detection of the sensory characteristics of food. Indeed, smelling, tasting and swallowing food is the first step to evaluate its energy and qualitative composition. Thus, we may wonder whether food intake regulation is based exclusively on its orosensory characteristics. To avoid the influence of taste and texture of the experimental diets on food selection, a feasible methodology is that of packaging different diets into a tasteless gelatine capsule. This new approach has allowed us investigate the influence of post-absorptive metabolic effects on food selection in different fish species (Rubio, Sánchez-Vázquez & Madrid 2003; Almaida-Pagán, Rubio, Mendiola, De Costa & Madrid 2006). In short, there is an evidence for post-ingestive signals (macronutrient release in the stomach, its subsequent passing through the intestine and detection by gastrointestinal receptors that activate nervous and/or endocrine pathways), regardless of diet organoleptic properties, which prompt brain centres that control food intake and diet selection. In this review, several studies on the effects of oral administration of different substances and food intake regulation using capsule-fed fish will be presented to further support this statement.

Figure 1 summarizes the central appetite regulators in fish and interrelations between them. An influence of environment and peripheral regulators complements the picture.

The biological clock that drives the feeding rhythm

Feeding rhythm is endogenously generated by an internal timing mechanism or biological clock. Thus, as one may expect for a true clock, in the absence of external time cues, circadian rhythms persist – free-run – with its own period, deviating slightly from the environmental cycle to which they are normally synchronized. Self-sustained rhythms usually persist for many days without attenuation, contrasting with hourglass-driven rhythms that disappear quickly as soon as external cues cease. For instance, if the feeding rhythm depended exclusively on hourglass physiological processes such as gastric emptying or metabolic rate, feeding would stop under fasting conditions. However, demand-feeding activity does not diappear; hence, fasted animals keep demanding for food at their preferred time (Madrid *et al.* 2001). The circadian system of fish is composed of a central pacemaker within the brain and at least two peripheral oscillators located in the retina and the pineal organ. The organization of such a system is basically comparable with that of mammals and birds, although some significant differences exist. For instance, the pineal organ of fish is a direct photoreceptor, which contains a circadian oscillator that controls the rhythmic secretion of melatonin (Mel) and transduces night length into Mel rhythms (Ekström & Meissl 1997; Falcón, Besseau, Sauzet & Boef 2007). This hormone is directly involved in many rhythmic physiological processes (e.g. reproduction and development) and behaviour (e.g. feeding, locomotor activity).

The circadian system of fish and other vertebrates is usually represented by a 'master' central pace-

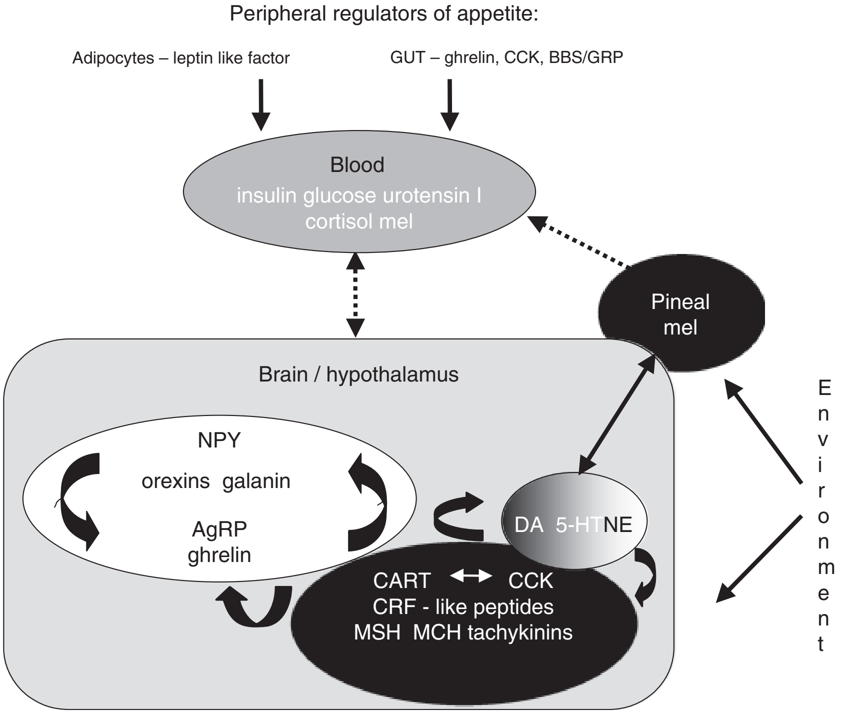

Figure 1 Appetite regulators in fish brain. Orexigenic factors (black letters on white): NE (norepinephrine), NPY (neuropeptide Y), orexins, galanin, AgRP (agouti-related protein) and ghrelin. Anorexogenic factors (white letters on black): CCK/gastrin (cholecystokinin – gastrin), CART (cocaine and amphetamine-regulated transcript), MCH (melanin-concentrating hormone), MSH (melanocyte-stimulating hormone), tachykinins, melatonin (Mel), DA (dopamine) and 5-HT (serotonin). Arrows, interactions between regulators and the influence of the environment and peripheral factors. Dotted lines, interactions via the circulatory system.

maker, which acts as a synchronizer of a wide variety of biological rhythms. Nevertheless, the existence of one or few pacemakers has been discussed in the last few years, suggesting that a basic timing mechanism is widespread in the organism, and so each cell may have its own functional circadian clock. Recent research on molecular genetics revealed that this clock is orchestrated by several clock genes, which express transcription factors that modulate its own expression. In fish, six *cryptochrome*, three *clock* and *bmal*, and probably four *period* genes have been described so far (for a review, see Vallone, Lahiri, Dickmeis & Foulkes 2005). These genes show interlocking transcriptional–translational feedback loops that encode valuable timing information. Light, temperature and feeding inputs act by setting in phase the rhythmic expression of clock genes with entraining cycles to enable fish to anticipate and respond properly to changes in their environment.

As discussed above, daily feeding rhythms may show considerable flexibility in some fish species, such as sea bass, which change their diurnal/nocturnal feeding behaviour. Food availability restricted to the daytime or the night-time could make a nocturnal fish diurnal and vice versa. However, not all fish react in this way, pointing to the existence of another factor decisive for the phasing of feeding rhythms. Apparently, sea bass show dualism under 'in doors' conditions, regardless of an artificial photoperiod and water temperature (Aranda, Sánchez-Vázquez & Madrid 1999). That is, fish do not respond passively to the manipulation of these environmental factors. However, in another study performed 'out doors' under natural conditions for a whole year, Sánchez-Vázquez, Azzaydi, Martínez, Zamora and Madrid (1998) observed a seasonal phase inversion phenomenon: sea bass feeding rhythms were typically nocturnal (main peak after dusk) during winter, and diurnal during the rest of the year. This finding indicates that some sort of gradual, progressive change of photoperiod and water temperature is required to trigger the phase inversions of feeding rhythms. Taken together, these results suggest that an endogenous circa annual clock is most likely involved in the control of the diurnal/nocturnal feeding behaviour of this fish species.

Feeding entrainment and feed intake control

Meal cycles have a profound effect on fish behaviour and physiology. If food is provided everyday at the same time, fish will synchronize to mealtime and develop increased activity in anticipation of the forthcoming meal. This phenomenon is known as feeding anticipatory activity (FAA), while feeding entrainment is related to the internal process, which couples meal cycles and rhythmicity. Regardless of the lighting conditions, the behavioural pattern of fish fed a single daily meal is characterized by a peak of activity a few hours before feeding-time, while the activity pattern of fish fed at random hours is flat and arrhythmic. Feeding entrainment persists during total food deprivation (fish maintain their activity around the previous mealtime), which indicates the existence of an endogenous circadian oscillator controlling food anticipation (for a review, see Sánchez-Vázquez, Aranda & Madrid 2001).

Timed food restriction is a prerequisite for the expression of FAA; when food is constantly available, fish obviously do not need to anticipate meals. In addition, a certain degree of food shortcut is required for the expression of FAA. Signals directly related to feeding, both pre-ingestive (e.g. foraging activity) and post-ingestive (gut distension and nutrient absorption/metabolism), are used for feeding entrainment. An almost empty stomach appears to be a stronger synchronizing signal than a full one, as a reduction in the meal size, but not in the dietary energy provided, significantly improves feeding entrainment (Sánchez-Vázquez *et al*. 2001).

Time-restricted feeding schedules act as time cues for temporal integration of daily rhythms. Thus, if food is limited to certain times, most fish will reset their internal clock and synchronize to mealtime. However, some species with more rigid feeding rhythms may not change their pattern of behaviour in response to 'unnatural' feeding schedules. For example, tench (*Tinca tinca*) feed exclusively at night, and so their activity rhythm remains strictly nocturnal irrespective of whether food is supplied during daytime or at night (Herrero, Madrid & Sánchez-Vázquez 2005).

It is known that seasonal and circadian changes in the environment can influence not only feeding behaviour but also dietary selection in fish. In goldfish, in which feeding behaviour is not confined to the light or the dark phase, there are strong timing preferences for macronutrients: carbohydrate during the light phase, protein during the dark phase and fat in the transition phase (Sanchez-Vazquez, Yamamoto, Akiyama, Madrid & Tabata 1998), which shows that light is a crucial external regulator of feeding preferences in fish. Indeed, diet choice is, thus, not random or purposeless, but it is directed to meet the particular nutritional requirements of fish. Fish will ingest a certain macronutrient at a certain time of the day based on its beneficial metabolic and physiological effects and will avoid those with harmful or less positive consequences.

In mammals, when food is freely available, a light signal entrains the circadian rhythms to the day–night cycle via the central oscillator in suprachiasmatic nuclei (SCN). However, in restricted-feeding animals, the food-entrainable clock operates independently and can even override the SCN to enable finding food (Fuller, Lu & Saper 2008). However, the anatomical location of the postulated food-entrainable oscillator (FEO), even in well-studied mammals, is still unknown (Davidson 2006).

In fish, the pineal organ, producing Mel at night, is a main component of the circadian system and biological calendar, which controls rhythmic function and behaviour. Retinal Mel is considered, rather, as an autocrine or a paracrine signal. Melatonin, mainly of pineal organ origin, is released into the circulation and the cerebrospinal fluid and regarded as an internal synchronizer. However, it is not clear whether the light-entrainable oscillator (LEO) of pineal and photoperiod-linked changes in the production of Mel in the pineal organ are involved in the regulation of feed intake. The pineal organ in fish can act as one circadian pacemaker in a multioscillator circadian system. In rainbow trout, for instance, the pineal organ does not seem to be a central pacemaker that controls the feeding rhythm, because the removal of the pineal organ does not disrupt the daily feeding rhythm (Sanchez-Vazquez, Iigo, Madrid & Tabata 2000). In goldfish, just a scheduled feeding has been shown to act as a Zeitgeber (Vera, De Pedro, Gomez-Milan, Delgado, Sanchez-Muros, Madrid & Sanchez-Vazquez 2007). The FEO is responsible for FAA. The existence of FEO has been presented in goldfish, sea bass, catfish and rainbow trout (Sanchez-Vazquez, Madrid, Zamora & Tabata 1997; Bolliet, Aranda & Boujard 2001; Vera *et al.* 2007); however, its anatomical location is unknown. Some reports suggest that NPY may be a signal of anticipation in the hypothalamus, at least in goldfish (Vera *et al.* 2007). The anticipatory secretion of amylase and hypothalamic NPY was observed 2 h before feeding, but only in fish fed periodically. The anticipation is controlled endogenously, because it persists after two days of fasting. In goldfish, maintained on a daily scheduled feeding regime, an increase in the NPY mRNA level in the telencephalon-preoptic region and in the hypothalamus shortly before feeding has been demonstrated (Narnaware *et al.* 2000). However, in general, anticipated feeding is scarcely explored in fish. Further, the relationship between FEO, light-entrainable pineal oscillator and neurohormones controlling feeding remains an unanswered question.

In fish, like in other vertebrates, there are extrapineal and extraretinal sites of Mel synthesis, i.e. GIT (Kulczykowska, Kalamarz, Warne & Balment 2006; Fernandez-Duran, Ruibal, Polakof, Ceinos, Soengas & Miguez 2007). The synthesis of Mel by the enterochromaffin cells of the intestine depends on the presence of food in the GIT, not on the photoperiod. Thus, a periodic feeding, not a photoperiod signal, can act here as a potent Zeitgeber. Therefore, the cycle of food availability and the presence of nutrients in GIT may be an important signal for the circadian system in fish. Whether GIT Mel is involved remains an open question.

Several studies have shown that increasing the photoperiod may result in an increased appetite in fish (Taylor, North, Porter, Bromage & Migaud 2006). Moreover, Mel may serve as an anorexigenic factor in many fish species such as goldfish, tench and sea bass (Pinillos, De Pedro, Alonso-Gomez, Alonso-Bedate & Delgado 2001; Rubio, Sanchez-Vazquez & Madrid 2004; Lopez-Olmeda, Sanchez-Vazquez & Madrid 2006). In goldfish, the GIT Mel level is high after feeding, regardless of the schedule or random feeding, but plasma Mel does not change (Vera *et al.* 2007). Also, in goldfish, intracerebroventricularly (i.c.v.) injected Mel and its agonists 2-iodomelatonin have no effect on feed intake. Thus, at least in goldfish, there is no evidence that Mel produced in GIT, contributes to a pool of circulating hormone, and as that, constitutes the signal for the hypothalamic centre to inhibit feeding. However, also in goldfish, the intraperitoneal Mel and its agonist injections have been proven to be effective in inhibition of feed intake (Pinillos *et al.* 2001). This effect is blocked by intraperitoneal administration of its antagonist, luzindole. A postprandial increase in GIT Mel, observed in this study, may be considered to be an anorectic peripheral signal in the feeding regulation system (Pinillos *et al.* 2001). On the other hand, in European sea bass (*Dicentrarchus labrax*), orally administered Mel, which evidently reaches peripheral circulation, can reduce the amount of food consumed in a dose-dependent manner (Rubio *et al.* 2004). Thus, in this species, besides Mel local action in GIT, its central anorexigenic signalling is postulated. It is suggested that the anorectic effect of Mel may be coupled with that of 5-HT (Rubio, Sanchez-Vazquez & Madrid 2006). Nevertheless, the orexigenic/anorexigenic effect on Mel administration may depend on the daily pattern of behaviour of fish, because Mel inhibits food intake in goldfish (a mostly diurnal species), but has no effect in tench (strictly nocturnal species) (Lopez-Olmeda *et al.* 2006).

Serotonin, a precursor of Mel, is known to inhibit feed intake in goldfish, rainbow trout and sea bass. The i.c.v. administration of 5-HT inhibits feed intake in goldfish, but no effect is observed after an intraperitoneal injection of the hormone (De Pedro, Pinillos, *et al.* 1998). The administration of fenfluramine, a 5-HT-releasing agent, induces a short-time inhibition of feed intake in rainbow trout, which probably is linked to the increasing availability of 5-HT in the serotoninergic system in the brain (Ruibal *et al.* 2002). On the other hand, in European sea bass, oral administration of 5-HT affects feed intake (Rubio *et al.* 2006).

Thus, central, peripheral or both mechanisms of 5-HT action should be considered in different fish species. Serotonin in fish, similar to that in mammals, may interact with other regulatory hormones such as insulin and CCK (Rubio *et al.* 2006).

Moreover, both 5-HT and Mel are involved in macronutrient selection in fish. In sea bass, orally administered 5-HT increases protein and reduces fat selection (Rubio *et al.* 2006). Also, orally administered Mel modifies the pattern of macronutrient selection in this species, with reduced carbohydrate intake (Rubio *et al.* 2004). The mechanism by which Mel and 5-HT reduce feed intake and influence selection of nutrients, still remains unknown, but some data suggest that they may be coupled with NPY regulation.

Figure 2 presents the factors that can affect the entrainment of feeding rhythm in fish. The relationships between various elements are shown.

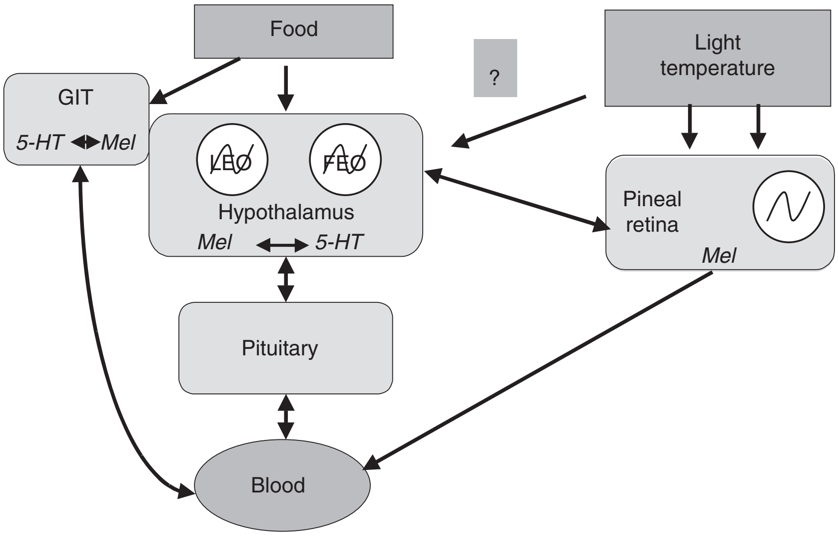

Figure 2 Feeding rhythm entrainment in fish. Light-entrainable oscillator (LEO); Food-entrainable oscillator (FEO); melatonin (Mel); serotonin (5-HT); and gastrointestinal tract (GIT). Arrows, interactions between regulators.

Stress response, neurohormones and feed intake control

In aquaculture, fish experience a variety of adverse conditions, i.e. crowding, handling, disturbing, confinement, aggression within the group, poor quality of water and scheduled feeding. They react to these challenges with a series of neuroendocrine adjustments, known as the stress response. Many forms of environmental, social and physical stresses inhibit feed intake and affect feeding behaviour, i.e. food searching, finding and capture. Appetite is one of the easily measurable indices of fish well-being, but the mechanisms of its control in fish subjected to different stressors are not clear.

The primary response to stress in fish involves two major neuroendocrine pathways: the hypothalamus–autonomous nervous system–catecholamine-producing chromaffin cells and the hypothalamus–pituitary–corticosteroid-producing interrenal cells. Corticotropin-releasing factor and UI-expressing cells of the POA and caudal neurosecretory system are considered to be key contributors to the regulation of the stress response in fish (Bernier, Alderman & Bristow 2008). There is a growing body of evidence indicating that both CRF and UI may be the endogenous mediators of the stress-induced inhibition of feed intake in fish (Bernier & Peter 2001a; Bernier 2006). In goldfish, an i.c.v. injection of CRF elicits time- and dose-dependent inhibition in feed intake and thus suggests that CRF may play a role in the central regulation of feeding (De Pedro, Alonso-Gómez, Gancedo, Delgado & Alonso-Bedate 1993). Intracerebroventricular injections of both CRF and UI, also in goldfish, show a dose-dependent inhibition in feeding, with UI being more potent (Bernier & Peter 2001b).

Different environmental and social stresses have been shown to result in CRF mRNA increases in POA of rainbow trout (Bernier *et al.* 2008). Corticotropin-releasing factor fibres secrete their contents close to corticotrophes in the neurohypophysis, in which adrenocorticotropin (ACTH) is produced from its precursor – proopiomelanocortin (POMC). Adrenocorticotropin, in turn, stimulates cortisol secretion from interrenal cells. Evidence exists that also reveals that the POMC-derived peptides from melanotrophs of the neurointermediate lobe of pituitary, MSH (melanotropin–MSH)- and endorphin-related peptides, can respond to stress (Flik, Klaren, Van den Burg, Metz & Huising 2006). Moreover, the stress response of corticotrophes and melanotrophs may be dependent on the type of stressor (Mosconi, Cardinaletti, Carotti, Palermo, Soverchia & Polzonetti-Magni 2006). *In situ* hybridization studies have demonstrated the expression of POMC mRNA in the hypothalamic areas associated with neuroendocrine regulation of feed intake (Cerdá-Reverter, Schioth & Peter 2003). Potentially, CRF-related peptides and melanocortins may be implicated in a wide variety of appetite-regulating pathways. It appears that melanocortin peptides can participate in appetite regulation in goldfish as anorexigenic factors (Cerdá-Reverter & Peter 2003;

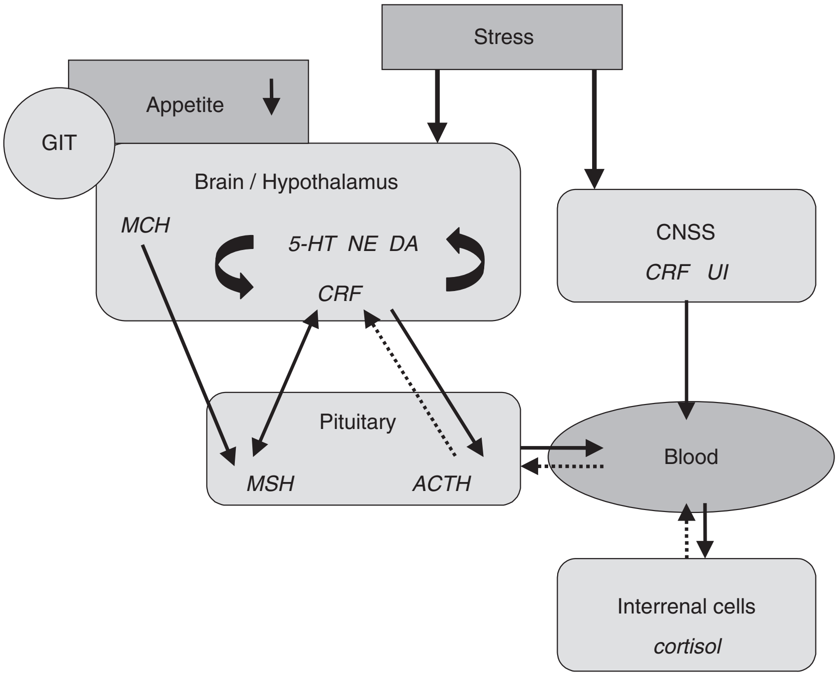

Figure 3 Stress response and appetite regulators in fish. Corticotropin-releasing factor (CRF), adrenocorticotropin (ACTH), urotensin I (UI), NE norepinephrine (NE), dopamine (DA), serotonin (5-HT), melanin-concentrating hormone (MCH), melanocyte-stimulating hormone (MSH), gastrointestinal tract (GIT) and caudal neurosecretory system (CNSS). Arrows, interactions between regulators.

Cerdá-Reverter *et al.* 2003; Shimakura *et al.* 2008). An intracerebroventricularly injected α-MSH agonist causes an inhibition of feed intake in goldfish (Cerdá-Reverter *et al.* 2003). However, an i.c.v. administration of β-endorphin has been shown to stimulate feeding in goldfish (De Pedro, Delgado & Alonso-Bedate 1995). It is not clear how POMC processing is regulated in response to different nutritional status of fish. It is also not resolved whether these peptides interact with other neuroendocrine signals in the brain involved in feeding control, or whether they act independently. Probably, melanocortins' anorexic effect is flattened by the competing binding of AgRP to the melanocortin receptors (Cerdá-Reverter *et al.* 2003). Hypothalamic expression of AgRP in goldfish is known to be up-regulated by fasting (Cerdá-Reverter & Peter 2003). The MSH feedback mechanism coupled with the leptin–regulation pathway has also been suggested (Flik *et al.* 2006). In Fig. 3, the hypothesized pathways of CRF-related peptides and melanocortins action are shown.

In fish, like in other vertebrates, the monoamine neurotransmitters 5-HT, DA and NE play an important role in response to stresses, which are associated with behavioural changes. For instance, rainbow trout subjected to social stress responded with a low feed intake. Increased release and turnover of 5-HT, DA and NE and their metabolites presented in these fish brains indicated high neural activity (Øverli, Winberg & Pottinger 2005). Thus, the central action of monoamine neurotransmitters has been included in the scheme of appetite regulation in fish subjected to stress (Fig. 3). Moreover, functional interaction between CRF and the hypothalamic catecholaminergic system in the central regulation of feed intake has been reported. In goldfish, i.c.v. administration of CRF reduced feed intake and hypothalamic NE and DA contents. These effects were reversed by α-helical $\text{CRF}_{(9-41)}$ pre-treatment (De Pedro *et al.* 1997). The anorectic effect of CRF-like peptides most probably was mediated by α_1-adrenergic and dopaminergic receptors (De Pedro, Delgado, *et al.* 1998). In tilapia, NA and 5-HT stimulated the release of CRF from telencephalic tissue in *in vitro* studies (Pepels, Wendelaar Bonga & Balm 2004).

The mechanism of action of CRF-like peptides may also include their inhibitory effect on orexigenic peptides and mediation of some effects of anorexigenic factors, similar to that reported in mammals (Heinrichs & Richard 1999). However, for fish, the data are still scarce. The distribution of a galanin-like peptide in the brain and pituitary of some fish species suggests that it could play a role of a neurotransmitter and/or a neuromodulator of ACTH secretion (Olivereau & Olivereau 1991). Thus, the interaction between the HPI axis and the orexigenic factor, galanin, if only could be supported in experiments, might be an example of such a mechanism.

In response to social stressors, other stress mediators are also implicated, e.g. 5-HT. The relationship between social status and brain serotonergic activity was studied in various fish species. In Arctic char (*Salvelinus alpinus*), feed intake was completely inhibited in subordinate fish, in which brain serotonergic activity, as indexed by the ratio of 5-hydroxyindoleacetic acid to 5-HT, was elevated compared with the dominants. Rearing the fish in isolation reversed the appetite inhibition, while the serotonergic activity of the brain was declining compared with the dominants (Øverli, Winberg, Damsård & Jobling 1998). The central anorectic action of 5-HT may be, at least in part, mediated by CRF in goldfish, because the reduction in feed intake caused by an i.c.v. injection of 5-HT is partly reversed by the injection of the CRF receptor antagonist, α-helical CRF$_{(9-41)}$ (De Pedro, Pinillos, *et al.* 1998). The 5-HT action can also be coupled with that of Mel (Rubio *et al.* 2006).

An increased growth of many fish species in aquaculture is associated with applied continuous or prolonged light regimes. However, an unnatural photoperiod, especially continuous light, is considered to be very stressful and in the long term, even as a life-threatening condition. Disruption of natural day–night rhythmicity by photoperiod manipulation has an adverse effect on the well-being of farmed fish. It affects the LEO of pineal or photoperiod-linked changes in the production of Mel in the pineal organ and implicates feeding. The response to unnatural lighting can also be considered in terms of response to stress.

It is taken into account that inappropriate feeding schedules may also be harmful for fish. Actually, feeding is a key factor when considering fish welfare (Huntingford, Adams, Braithwaite, Kadri, Pottinger, Sandøe & Turnbull 2006). Moreover, fasting, as well as other stressors such as handling and hypoxia, are known to increase plasma cortisol, metabolites (i.e. glucose and lactate) and to trigger the release of catecholamines in many teleosts (Barton & Iwama 1991). Although food intake is regarded as a reliable criterion to evaluate fish welfare under different experimental and farming conditions (Turnbull, Bell, Adams, Bron & Huntingford 2005), to date, the effect of feeding time on fish welfare remains little explored. Recent investigations, however, pointed out that the feeding cycle considerably influenced growth performance, fish behaviour, cortisol and glucose levels in gilthead sea bream. The random-fed (RF) fish showed a lower growth rate and higher cortisol and glucose levels than fish that received food at a scheduled time (SF). Moreover, 10-fold higher cortisol values in the RF group than that in the SF group indicated that fish of the RF group were under stress (Sánchez, López-Olmeda, Blanco-Vives & Sánchez-Vázquez 2009). In addition, fish fed every day at the same time showed increased secretion of amylase and NPY two hours before feeding, whereas fish fed randomly did not show anticipation in any digestive or endocrinal factors (Vera *et al.* 2007). Moreover, randomly fed fish are forced to increase their locomotive activity to keep up a continuous alert to avoid missing food. As a result, plasma cortisol is higher in randomly than in scheduled fed fish, suggesting that the lack of a meal cycle is stressful and compromises fish welfare. Indeed, FAA is thought to be of adaptive value. From a physiological and behavioural point of view, while a continuous active state is uneconomical, anticipation to mealtime is highly beneficial.

The effects of feeding time on food intake and growth of farmed fish are well-known, and good knowledge of fish feeding behaviour is the basis for improving feeding management in aquaculture: if meals are programmed to match fish appetite, food utilization is expected to be maximal. Because the appetite of fish may fluctuate during the day, meals should be modulated accordingly. If food delivery is distributed in several meals, adjusted in accordance with the feeding rhythms of fish, rather than equally sized meals, growth performance could be improved (Azzaydi, Martínez, Zamora, Sánchez-Vázquez & Madrid 1999). Furthermore, because feeding rhythms may shift from diurnal to nocturnal depending on the season, a sensible feeding strategy must bear in mind such changes. In winter, sea bass feeding behaviour is strictly nocturnal; thus, fish fed at night grew significantly quicker than those fed during the daytime (Azzaydi, Martínez, Zamora, Sánchez-Vázquez & Madrid 2000).

In summary, feeding rhythms and appetite regulation are model examples of how a variety of neurohormones interact in order that nutritional demands can be fulfilled and energy balance can be achieved according to the feeding habits of the fish. This complicated system is very sensitive to any disturbance.

Fish in farms and fish in natural environment are equipped with the same combination of neurohormones to regulate feed intake, but they meet different challenges. Finding the best time to deliver food is not an easy task, as fish may change their feeding behaviour at certain times. Nevertheless, the study of feeding rhythms and FAA may provide useful information to design improved feeding schedules that match fish appetite. It is suggested that hypothalamic 5-HT and CRF-like peptides may play an important role in the regulation of feed intake in farm fish subjected to stresses.

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