Influences on fish pigmentation

- Pigmentation: Melanotropic axis

Skin colouration is one of the most striking features of the teleost phenotypes. Fish pigmentation is a plastic feature that can be adjusted to according to fish needs: e. g. social interactions (Grosenick *et al.* , 2007), camouflage (predator avoidance) (Doolan *et al.* , 2009) or mating selection (Grether *et al.* , 2005). Pigmentation (colour and pattern) is determinate by the amount, type and distribution of the pigment cells (chromatophores), delimitated by the genotype, species, gender and developmental stage. Changes in skin colouration can be morphological (changes in the intracellular pigment material) or physiological (pigment motility within chromatophores). The latter can be: primary (caused by environmental factors: e. g. light) or secondary (nervous and endocrinal translocations: e. g. ACTH and alpha-melanocyte-stimulating hormone, α-MSH). Physiological colour changes are virtually instantaneous (visible within minutes or hours) caused by movements of pigment vesicles or reflective structures within their respective cell (Leclercq *et al.* , 2010). Morphological changes are slower and have a long lasting effect on the colouration. They are driven by changes in the skin pigment density and distribution (expand or contract) due to environmental stimuli (proximate) or as part of the phenotype transition between developmental stages (ultimate) (Leclercq *et al.* , 2010).

- Chromatophores

Chromatophores are specialised cells that storage and synthesise (light absorb or reflect) pigment structures. Based on their light sensitivity they are categorised in: melanophores (black), erythrophores (red), xanthophores (yellow), iridophores (silver) and leucophores (white). In flatfish only three

types of chromotophores are present: melanophores, xanthophores and iridophores (Bolker and Hill, 2000). They originate during early embryo ontogeny from neural crest cells (Bolker *et al.*, 2005). During the embryonic and early larvae stages stem cells migrate symmetrically on both sides. During metamorphosis cells undergo a second differentiation producing additional melanophores and complement pigment cells, which results in a blind side and the ocular side pigmentation pattern (Seikai *et al.*, 1987). The melanophores and xanthophores are dendritic cells that translocate light-absorbing pigment granules and store melanins and pteridines, respectively. Iridophores are not dendritic, but rather stacked refractosomes (transparent reflecting purine) with a non-fixed structure and organisation (colour reflectance depends on the angle of observation) (Menter el al. 1979).

Flatfish pigmentation patters respond easily to proximate stimuli, for example the rapid colour match from the upper side to that of the background (Healey, 1999). Light perception plays a crucial role allowing the dispersion or concentration of melanophores to match the colouration of the environment. Furthermore melanin synthesis in the chromatophores initiates in flatfish before metamorphosis is accomplished by the regulation of MSH form the pituitary (Suzuki *et al.*, 1998). Light intensity (and possibly spectral composition) play a crucial role in the initiation of the pigment synthesis and remain so during the adult form. Incidences of abnormal pigmentation (albinism or bicolouration) in flatfish larvae are increased by light intensity (darkness or high intensity) during rearing (Venizelos and Benetti, 1999). However partially albinic colouration can be corrected (prior the end of

metamorphosis) when light intensity is adjusted closer to the optimal (Denson and Smith, 1997).

- Involvement of the visual system

Since the early studies in the 19 [th] century it became clear that vision plays a crucial role in pigmentation control. Despite not being able to gain access to the original publications, Henslow (1872) highlighted in a letter to Nature that the work of Pouchet (1872) demonstrating that skin colouration mimicking background in prawns was prevented by removing their eyes, required more attention that the given at the time. Afterwards Pouchet (1876) further demonstrated that turbot skin was darken by sectioning the peripheral nerves and whiten (pale colouration) by electrical stimulation of the spinal nerves (cited in Scott, 1965). More recently Kanazawa (1993) found that abnormally pigmented Japanese flounder (*Paralichthys olivaceus*) had no preference between light (exposed) and dark (covered) tank compartments, while normally pigmented would gather in the dark section. The author suggested that the differences in background preference were due to an impaired visual system in the malpigmented fish. Supplementation of vitamin A and fatty acids (particularly: docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) in the diets improved pigmentation, likely driven by retinal rhodopsin formation that ultimately leads to melanin production. Vitamin A is a group of morphogenetic nutrients that fish are not able to synthetize and need to be absorbed though the diet. The active retinal form is used as the chromatophore of rhodopsins (Pepe, 1999), while other forms are involved in a range of functions: e. g. skeletal tissues

development (Fernández and Gisbert, 2011) and immune condition (Dhert *et al.* , 1994).

- Involvement of nutrition

Essential fatty acids are also involved in a whole range of functions, being critical for the nervous system development during the larval stages (Hamre *et al.* , 2013). Nutritional deficiencies in the artificial diets are linked to a higher incidence of abnormal pigmentation in aquaculture (Bolker and Hill, 2000). Turbot fry fed artificial diets rich in DHA achieved the highest pigmentation levels (Dhert *et al.* , 1994), however it has been suggested that the ratio between DHA to EPA is the key factor, rather than absolute fatty acid levels (Dhert *et al.* , 1994; Sargent *et al.* , 1997; Hamre *et al.* , 2013). Furthermore, turbot larvae fed live-prey enriched with high doses of arachidonic acid (ARA) and EPA resulted in a higher incidence of abnormal pigmentation (Estevez *et al.* , 1999). The variance between results suggests that nutrition plays a crucial role in determining pigmentation success. However it is clear that pigmentation does not depend solely on the proximate factors but rather on their neuroendocrinal effect.

- Physiology of pigmentation

The two major hormonal peptides associated in physiological pigmentation changes are melanocyte-stimulating hormone (MSH) and melanin-concentrating hormone (MCH). MSH is produced in the neurointermediate lobe of the pituitary gland derived from proopiomelanocortin (POMC) and stimulates pigment dispersion in the chromatophores (skin darkening). MCH is produced in the hypothalamus and secreted by the pineal gland through neural fibres and induces pigment aggregation (skin lighten). The opposing

functions between MSH and MCH in pigment migration are clearly evident *in vitro* (Baker, 1993; Burton and Vokey, 2000). However *in vivo* MSH does not necessarily disperse pigments, because interrelation between them is masked by the dominant neural effects (Mizusawa *et al.*, 2013). Five melanocortin receptors (MCR) subtypes (MCR1 – MCR5) have been cloned in tetrapods, highly homologous in lower vertebrates. The number of receptors diverges between fish species (Table X): e. g. zebrafish possess six (two copies of MCR5) and pufferfish lacks MCR3.

- Interactions beteween Corticotropin, Melanotropin and Thyrotropic axis

MCR regulate melanogenic activity thought its' high affinity to α-MSH but also to ACTH (Selz *et al.*, 2007). MSH (α-, β, γ-MSH) shares POMC as the common precursor with the ACTH (Metz *et al.*, 2006). Fig. X integrates the major factors affecting the melanotropic and corticotopic axis in fish. It is important to mention the contribution of the melanotropic axis on the endocrinal stress response is species specific and depends on the nature and intensity of the stress stimuli. For instance, in sea bream (*Sparus aurata*) air exposure (3 min) resulted in increased plasma levels of cortisol, α-MSH, glucose and lactate; but ACTH and βEND were maintained. Stress by confinement (70 Kg m $^{-3}$) triggered a rapid increase (1 h) of cortisol, ACTH and α-MSH (Arends *et al.*, 1999). Plasma α-MSH differed based on background adaptation (highest in the white background), but plasma cortisol levels were similar between treatments (Arends et al, 2000). Similarly, white background increases aggressive behaviour and skin darkening in Arctic charr (*Salvelinus alpinus*) (Höglund *et al.*, 2002). The skin darkening was firstly correlated to a stress-induced reaction due to the

higher plasma levels of α-MSH, ACTH and cortisol (Höglund *et al.* , 2000). It appears that multiple interactions interconnect the corticotropic and melanotropic axis. However available information is scarce or contradictory, mainly due to the different experimental approaches, species analysed and the still undefined targets and functions of those hormones. For instance, skin darkening in sole was correlated to cortisol levels bur not α-MSH (Ruane *et al.* , 2005).

- Endocrine regulation of feed intake

Food intake regulation is a complex process that involves central and peripheral factors, stimulated by environmental signals, energy reserves, metabolic rate, developmental stage and reproductive status. Food intake regulation has been highly preserved across vertebrates considering the physiological differences between taxa. Moreover the indeterminate growth of fish results in a continuous resource allocation between reproduction and growth. The sole role of food intake regulation is to maintain the energy homeostasis through orexigenic (appetite stimulation) and anorexic (appetite inhibition) factors (Volkoff *et al* ., 2005). The brain (mainly the hypothalamus) and peripheral hormones regulate food intake, through signalling energy status of different systems. This added to the interaction between hormones and apparet shared functions of several orexigenic and anorexic factors, results in a complex neuroendocrine process that is still not fully understood. Neuropeptides interact closely with each other and in parallel. Figure 15 is a simplified representation of the regulation of appetite model in fish.

- Orexigenic factors

Neuropeptide Y (NPY) family consists on NPY, peptide YY (PYY), pancreatic polypeptide (PP) and peptide Y (PY). They regulate food intake in teleost modulating other appetite regulators. So far five receptors have been cloned: Y1, Y2, Y4, Y5 and Y6 (Larhammar *et al* ., 2001) widely distributed in the CNS, pituitary and gut (Volkoff *et al* . 2009). NPY is a strong orexigenic factor and levels undergo periprandial variations in several species: e. g. Atlantic cod (Kehoe and Volkoff, 2007). Orexins (OX-A and OX-B) are peptides produced in the brain including the hypothalamus, in pituitary and in peripheral tissues (i. e. gut) (Xu and Volkoff, 2007). Galanin (GAL) is produced also in the brain, pituitary and perfierial tissues (i. e. olfactory bulb in the goldfish) (Unniappan *et al* ., 2004). Agouti-realted protein (AgRP) physiological role in fish is unknown (Volkoff *et al* ., 2005), however in mammals AgRP is involved in the control of energy homeostasis and feeding. Furthermore the agouti-signalling protein (ASP) is a competitive antagonist of the melanocortin receptors: MCR1 and MCR4. Ghrelin is predominately secreted in by the stomach and into less extent in the brain. In mammals, ghrelin stimulates appetite (by activation/inhibition of AgRP, NPY and POMC neurons) and GH secretion (Olsewski *et al* ., 2008). The orexigenic function of ghrelin has been confirmed in some fish species (e. g. Miura *et al* ., 2009) and its interaction with other appetite peptides (NPY and OX) (Miura *et al* ., 2006 and 2007).

- Anorexic factors

Cocaine- and amphetamine-regulated transcript (CART I and CART II) peptides are present in the fish brain, pituitary and peripherial tissues (i. e. gonads and kidney) (Singru *et al* ., 2007). Human CART injection inhibit

feeding in goldfish (Volkoff and Peter, 2000), however orexigenic actions have also been reported in rats (Abbot *et al* ., 2001). CART inhibits both NYP and OX-A (Volkoff and Peter, 2000) and interacts with leptin (Volkoff *et al* ., 2003). Cholecystokinin (CCK) is structurally related to gastrin. CCK mRNA is detected in the brain, pituitary and intestine (Peyon *et al* ., 1999). It is released when food is present in the intestine, influencing digestion, feeding processes and inhibiting appetite (Volkoff *et al* ., 2003). Bombesin and gastrin-releasing peptide (BBS/GRP) regulate gut motility in several fish species (Bjenning *et al* ., 1991) and is expressed in the gastrointestinal tract, cardiovascular system and CNS (Himick *et al* ., 1995; Bjenning *et al* ., 1991). Glucagon-like peptide (GLP) is produced in the pancreas and intestine. GLP induces anorexia and causes gastric emptying (Plisetskaya and Mommsen, 1996). Leptin is mainly expressed in the fish liver, brain, pituitary and peripheral tissues (e. g. ovary of pufferfish) (Wong et al., 2007; Kurokawa et al., 2008). In most fish species, leptin decreases appetite and increases fat metabolism (Londeraville and Duvall, 2002). Tachykininis (TKs) is expressed in the CNS, it inhibits appetite and is involved in feeding and digestion processes (Peyon *et al* ., 2000)

- Interaction with the Melanocortin axis

In fish and mammals it appears that the MCR4 play a key role in the regulation of food intake and energy balance (Metz *et al* ., 2006). In zebrafish larvae, α-MSH and AgRP cells in the hypothalamus are more pronounced coinciding with the appearance of active feeding behaviour (Forlando and Cone, 2007). In goldfish feeding was inhibited by central injections of NDP α-MSH (MCR4 agonist) or MT II (MSH agonist), but does not modify mRNA

levels of POMC in the brain (Cerdá-Reverter *et al.* , 2003; Matsuda *et al* ., 2008). MCH involvement in food intake regulation remains controversial; however in mammals it is involved in energy homeostasis and feeding. In goldfish MCH reduces food intake and fasting decreases MCH-like ir neurons (Matsuda *et al* ., 2007). The process seems to be regulated through NPY and MSH but not CRF, PASCAP or CCK (Shimakura *et al* ., 2008; Matsuda *et al* ., 2009). Controversially barfin flounder reared in a white background presented enhanced body growth and greater expression of the MCH gene and number of MCH neurons (Takahashi *et al* ., 2004).

- Interaction with the Corticotropin axis

One of the additional involvements of the CRF system appears to be the regulation of food intake and energy balance, linked to some extent to the stress response (Richard *et al* ., 2002). CRF-related peptides play a role regulating appetite in fish though increased CRF mRNA in the forebrain (Bernier and Peter, 2001; Doyon *et al* ., 2003). Based on mammalian models it appears that CRF modulates gastrointestinal motility and gastric emptying (Wang *et al* ., 2001; Tache and Perdue, 2004), however the CRF-related peptides interactions with food intake regulation in fish are still in its infancy (Bernier *et al* ., 2004). Moderate chronic administration of cortisol stimulates food intake, by increasing NPY and decreasing CRF brain expression, while high doses does not impact food intake or NPY expression (Bernier *et al* ., 2004).

- Influence of light on feed intake

Feeding activity is regulated by photoperiod likely by increasing food intake and/or muscle mass by exercise (Boeuf and Le Bail, 1999). Melatonin

administration reduces food intake and body weight in goldfish, but does not affect plasma leptin, ghrelin or brain NPY levels (Pinillos *et al* ., 2001, De Pedro *et al* ., 2008). Studies on the effects of light intensity in growth are normally focused on fish growth in term of the visibility of the prey or food-stuffs and a direct implication in appetite stimulation has not been investigated (Bouf and Le Bail, 1999). In term of the light spectrum, to my knowledge only one study has reported the effects of different coloured lights in feed intake. Tilapias feed intake was enhanced when exposed to red light, while under blue light plasma cortisol levels were reduced and reproductive behaviour enhanced (Volpato *et al* ., 2001 and 2013).