Smoltification

**Sigurd O Stefansson[1],*, Björn Th Björnsson[2],
Lars OE Ebbesson[1] and Stephen D McCormick[3]**

INTRODUCTION

The Anadromous Life History Strategy

Several salmonid species, including members of the genera *Oncorhynchus*, *Salmo* and *Salvelinus*, are anadromous and undergo a significant structural and functional transformation prior to migration to seawater. This has been termed parr-smolt transformation or, as we will refer to, smoltification, and has previously been reviewed by Hoar (1976; 1988), Wedemeyer *et al.* (1980), Barron (1986), McCormick & Saunders (1987) and McCormick *et al.* (1998). Early biologists believed that the downstream migrating smolt was a distinct species of fish, but it was later discovered that it was the round, cryptically coloured, bottom-dwelling parr which, in the spring, transformed into a streamlined, silvery, pelagic smolt. During smoltification, the behaviour of the

Authors' addresses: [1] Department of Biology, University of Bergen, Bergen High Technology Centre, N-5020 Bergen, Norway.

[2] Fish Endocrinology Laboratory, Department of Zoology/Zoophysiology, Göteborg University, Box 463, S40530, Göteborg, Sweden.

[3] USGS, Conte Anadromous Fish Research Center, Turners Falls, MA 01376, USA.

Corresponding author: Department of Biology, University of Bergen, Bergen High Technology Centre, N-5020 Bergen, Norway. E-mail: sigurd.stefansson@bio.uib.no Tel: +47 55584470, Fax: +47 55584450.

juvenile salmon changes from territorial and bottom-dwelling to pelagic and schooling, a strategy which reduces risk of predation in open waters (Handeland *et al.*, 1996). Despite contrasting evidence of changes in swimming performance of parr and smolts (Virtanen & Forsman, 1987; Peake & McKinley, 1998), parr actively swim against the water current to keep position, while smolts develop increased buoyancy and a preference for moving downstream out of the river system. The biological significance of this behaviour is to enter the ocean at an appropriate time, avoid predation in the estuary, and migrate from coastal waters to marine feeding grounds offshore.

Smoltification consists of a number of independent, but co-ordinated developmental changes in the biochemistry, physiology, morphology and behaviour of the juvenile salmon. Alterations in lipid metabolism, osmoregulation, oxygen transport, buoyancy, growth, colour, shape, rheotaxis, and schooling behaviour can be understood as being preparatory to maximise the success of a life history strategy which involves downstream migration, ocean entry and subsequent long-distance feeding migrations in the marine environment. At the same time, many of these changes would be detrimental for continued life in freshwater, and it is thus not surprising that smoltification-related changes revert relatively quickly (desmoltification), if the fish are unable to enter the marine environment during the period of complete smoltification (the smolt 'window').

The anadromous strategy allows the different species to take advantage of the two very different ecosystems; the limnic, including rivers and lakes, and the marine, including both coastal areas and the open ocean. Reproduction is limited to rivers, an ecosystem which in temperate and arctic regions holds relatively few predators, thereby increasing the chances of survival during the early stages. The ecosystem is also characterised by low productivity, supporting only a limited growth rate and size of the fish. By leaving the river at a certain life stage, and entering the ocean to exploit the rich marine food supply, salmonids can increase their reproductive success ('fitness'). Models predicting the probability of an individual adopting an anadromous versus a resident strategy suggest that several factors including growth conditions, cost of migration, mortality rate and fecundity are involved (Gross, 1987). As will be discussed later in this chapter, residency is an evolutionary viable reproductive strategy for males, and in many anadromous populations a portion of the males do not migrate to the sea. On a global scale, anadromy is more common in temperate and sub-arctic regions, and is more widespread in the Northern than Southern hemisphere (McDowall, 1987). In contrast, catadromy is more common in the tropics and sub-tropic/warm temperate regions. Hence, in general terms, the frequency of anadromous and catadromous species reflect the relative productivity of the freshwater and marine environments.

Anadromy is not unique to salmonids. It is a strategy found among several fish groups both in the Northern and Southern hemispheres, including lampreys, sturgeons, osmerids, clupeids (shads) and basses (e.g. striped bass, *Morone saxatilis*). What sets the salmonids apart from other anadromous species is the smolt stage. Smoltification is a *preparatory adaptation* occurring in fresh water *before* the juvenile salmon experience saltwater. This contrasts with acclimation that is initiated due to exposure to a new or variable environment. Under natural conditions in rivers and lakes, smoltification is a

seasonal process usually occurring in late spring. Due to its similarity to metamorphosis (see Power *et al.*, this volume), smoltification was thought to be a single process, where the regulatory mechanisms for changes in morphology, physiology and behaviour were coupled. However, detailed studies have revealed that smoltification is a synchronisation of several independent developmental processes, and further, that certain processes may become uncoupled, e.g. the lack of correlation between salinity tolerance and body silvering (Bern, 1978). Manipulations which affect only certain components of smoltification can result in incomplete transformation and a reduced potential to exploit or survive in the marine environment. Such reduced adaptive potential may explain the limited success of hatchery-released smolts noted for different species during the last decades. There is evidence that the seasonal nature of smoltification has a basis in an endogenous rhythm, yet environmental cues are of major importance as *zeitgebers* for complete smoltification (Stefansson *et al.*, 1991; Berge *et al.*, 1995; Björnsson *et al.*, 1998; McCormick *et al.*, 2000). As will be discussed later, this interplay between endogenous and exogenous control of smoltification is mediated by the neuroendocrine system.

The age and size at which smoltification occurs and its relative intensity differs among salmonid species. In pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*), seaward migration takes place soon after emergence from the gravel, and almost the entire life cycle and all growth takes place in the sea. At the other end of the scale are the charr (*Salvelinus* spp.), which are entirely freshwater resident or spend only a few weeks feeding in seawater during the summer. Smoltification in Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Oncorhynchus mykiss*) occurs after at least one year residence in fresh water. A possible explanation for the heterochrony of salmonid smoltification is discussed by McCormick (1994). In this review we will focus on smoltification in Atlantic salmon, which is the most widely used salmon in aquaculture and has the largest body of scientific literature related to smolt development.

ALTERNATIVE LIFE HISTORIES

Desmoltification

Entry into seawater can be regarded as the manifestation of smoltification, a commitment to an oceanic life. However, if smolts are prevented from exposure to seawater, several of the preparatory changes associated with marine life are abandoned, a process known as desmoltification in Atlantic salmon or parr-reversion in Pacific salmonids (Hoar, 1988; Duston *et al.*, 1991). Accordingly, a 'smolt-window' exists, during which smolts can enter, and quickly adapt to, seawater. Desmoltification is accelerated by short photoperiod (Kurokawa, 1990) and by high and increasing freshwater temperatures (the smolt window closes again, Soivio *et al.*, 1988). The duration of the smolt window is suggested to be in the range of 300-400 degree-days (d°C; Stefansson *et al.*, 1998; McCormick *et al.*, 1999, see also Temperature below).

Desmoltification is generally viewed as a loss of important preparatory adaptations to marine life, including loss of hypo-osmoregulatory ability, critical metabolic adaptations, behavioural changes (e.g. re-establishment of positive rheotaxis) and major endocrine changes. Fish which enter seawater around or after the time of the closing of the smolt window generally show lower feed intake and growth, higher plasma ion levels, and reduced growth hormone (GH) levels, all indications of loss of seawater adaptability (Arnesen *et al.*, 2003). The desmoltification process is generally characterised by only a partial loss of morphological smolt characters, the fish again taking on a darker appearance, although not reverting to the morphology of parr, despite the loss of critical physiological functions. Evidence from culture of Atlantic salmon demonstrate that seawater growth of post-smolts is poor, disease resistance is lower and the fish often fail to adapt to harsh conditions (low temperature, high salinity) during winter.

Parr Maturation

According to Thorpe (1994) smoltification can be regarded as an alternative to the strategy of sexual maturation at the earliest opportunity (parr). Hence, some juvenile salmon become sexually mature in freshwater, without prior smoltification and seawater migration. Parr maturation is a viable strategy among male salmonids in many species and populations, and is generally observed when conditions for freshwater growth and development are favourable. On the other hand, maturation among female parr is normally not a viable strategy, as fecundity is drastically reduced (Hutchings & Jones, 1998). However, in cases of extremely low adult returns, due to high marine mortality or prevention of upstream passage, female parr maturation may be observed in salmonids as a response to the altered conditions and suggest the development of a landlocked life history. The basic mechanisms controlling the onset of puberty in fish, or indeed in any vertebrate, are still unresolved, although evidence points to a complex interaction between energy status, growth rate, size and genetics.

The process of sexual maturation involves major endocrine changes along the brain-pituitary-gonad axis. At the brain-pituitary level an increase in gonadotropic releasing hormone (GnRH) and a reduction in dopamine (DA) stimulate the synthesis and release of follicle stimulating hormone (FSH) and luteinising hormone (LH) from pituitary gonadotropic cells which in turn stimulate the production and release of sex steroids from gonadal tissues. Several points of interaction between the endocrine control of sexual maturation and smoltification can be identified, including a wide range of neuroendocrine factors, pituitary hormones and peripheral endocrine systems. Androgens have been shown to impair seawater adaptability (Lundqvist *et al.*, 1989), and downstream migratory activity (Berglund *et al.*, 1994) in smolting Atlantic salmon. Jakobsson *et al.* (1997) demonstrated receptor-like binding of androgens in Atlantic salmon gills, suggesting that the effect of androgens on osmoregulation may be exerted directly at the gill level, in addition to subsequent cascade systems. Madsen *et al.* (1997; 2004) and McCormick *et al.* (2005) found that estradiol (E2) and the synthetic

estrogenic compound 4-nonylphenol (4-NP) inhibited the progress of smoltification, as judged by an elevated condition factor and a significantly poorer hypo-osmoregulatory performance, that was correlated with a reduction of gill Na^+,K^+-ATPase (NKA) activity, relative a-subunit NKA mRNA expression and gill chloride cell density (see further discussion of ion/osmoregulation later in this chapter). McCormick *et al.* (2005) further reported decreased plasma insulin-like growth factor-I (IGF-I) and thyroid hormone (TH) levels, suggesting complex endocrine pathways for the actions of sex steroids and endocrine disruptors. Negative effects of E2 on salinity tolerance appears to be a general response among several species of teleost, as Vijayan *et al.* (2001) found reduced hypo-osmoregulatory capacity in E2 treated Mozambique tilapia (*Oreochromis mossambicus*).

In a recent study on maturing adult sockeye salmon (*Oncorhynchus nerka*) Shrimpton *et al.* (2005) described an overall decline in gill NKA activity and changes in NKA isoform mRNA expression from open ocean through coastal, estuarine and river migration. Although no endocrine data were presented, studies by Cooke *et al.* (2006) documented a concurrent increase in levels of sex steroids in maturing sockeye. Taken together, these data suggest one mechanism by which sex steroids may reduce seawater tolerance in maturing salmonids.

Landlocked Atlantic Salmon

It is argued that landlocked forms of Atlantic salmon are derived independently from various anadromous founder populations, which were later prevented from reaching the upper regions of watersheds due to the post-glaciation elevation of land (Power, 1958; Behnke, 1972). Populations of landlocked Atlantic salmon from North America and Europe differ in their capacity to adapt to seawater (Barbour & Garside, 1983; Chernitsky & Loenko, 1983; Burton & Idler, 1984; Birt & Green, 1986, 1993; Birt *et al.*, 1991; Staurnes *et al.*, 1992; Schmitz, 1995), although they seem to have retained a certain degree of silvering enabling them to adapt to a pelagic life in lakes (Dahl, 1928; Berg, 1953; Birt & Green, 1986; Nilsen *et al.*, 2003). Non-anadromous salmon from Five Mile Pond East stock (Avalon Peninsula, Newfoundland) show no smolt-related increase in gill NKA activity (Birt & Green, 1993) and are unable to maintain normal hydro-mineral balance when challenged with seawater (Burton & Idler, 1984). In contrast, landlocked 'Gullspång' Atlantic salmon from lake Vänern in south–western Sweden show a gradual increase in hypo-osmoregulatory ability during spring (Schmitz, 1995), although no information is available on changes in gill NKA activity nor long-term seawater adaptability. Studies of landlocked strains of Atlantic salmon in eastern North America have yielded somewhat contrasting results. The general pattern seems to be a gradual improvement in seawater tolerance during spring, despite lower levels of gill NKA activity than those found in anadromous populations (Birt & Green, 1986; 1993; Birt *et al.*, 1991).

Recent findings by Nilsen *et al.* (2003) show that the 'Bleke' population from lake Byglandsfjord, south-central Norway, having been isolated from the sea for more than

9000 years (Berg, 1985), have abandoned key elements of smoltification associated with long-term adaptation to seawater (Fig. 1). Despite still showing some euryhaline capacity and ability for short-term hypo-osmoregulation, Bleke show peak freshwater NKA activity at only 50% of levels observed in anadromous smolts and no preparatory changes in gill NKA isoform expression nor $Na^+,K^+,2Cl^-$ co-transporter (NKCC) or cystic fibrosis transmembrane regulator I (CFTR I) mRNA levels (Nilsen *et al.*, 2007). In a similar fashion, smolt-related development of critical brain regions and major endocrine changes are significantly attenuated in Bleke (Nilsen *et al.*, 2007; Ebbesson *et al.*, unpublished data). In line with the lack of preparatory changes in ion regulation, no smolt-related reduction in condition factor can be observed among landlocked populations, suggesting the absence of preparatory metabolic changes (Birt & Green, 1986; Birt *et al.*, 1991; Nilsen *et al.*, 2003). These differences may be viewed as adaptations to the generally poorer growing conditions of freshwater lakes compared with the North Atlantic Ocean. Smoltification is an energetically demanding process (Hoar, 1976; 1988), and in the case of landlocked populations aspects of smoltification associated with marine life may thus be considered maladaptive. The period that populations have been released from selection pressure on maintenance of physiological capacity for seawater tolerance may have resulted in the observed differences in salinity tolerance and other aspects of smolt development seen among landlocked populations of Atlantic salmon.

PHYSIOLOGY – PREPARATORY CHANGES FOR MARINE LIFE

Growth and Metabolism

Through the evolution of each species, the anadromous strategy has been favoured in regions where conditions have enabled salmon to utilise freshwater for reproduction, with juveniles migrating into the ocean to feed on the rich supply of fish, squid and crustaceans. Concurrent with the increase in hypo-osmoregulatory ability, other changes occur which are related to growth and metabolism and which prepare the juvenile salmon for the changes in availability and composition of prey organisms.

Growth pattern in freshwater

A major component of the anadromous strategy is for the smolts to enter the ocean at a size where risk of predation is significantly reduced. The schooling behaviour of smolts in the ocean is also advantageous for predator evasion. However, these strategies require that the juvenile salmon achieve a minimum threshold size, and that the smolts are fairly uniform in size. A primary observation of these relations is the occurrence of a bimodal size–frequency distribution that is related to smolt development (Kristinsson *et al.*, 1985). Depending on the overall conditions for growth and development, bimodality can be observed at the end of the first growing season in Southern populations (0+, Utrilla & Lobon-Cervia, 1999), whereas the same bimodal distributions may take
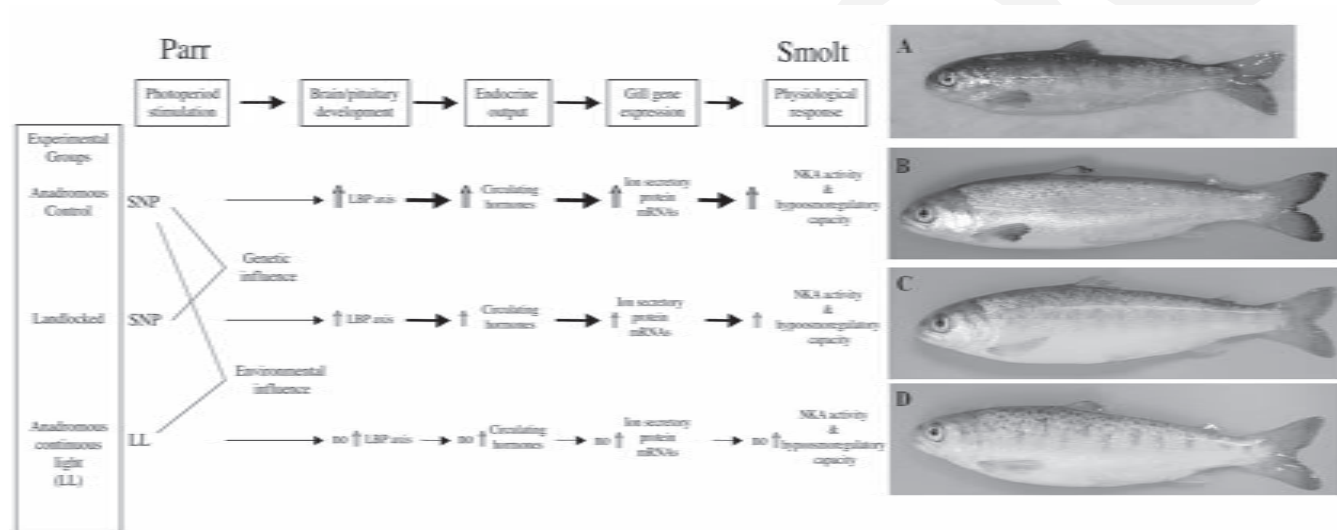
Fig. 1 This schematic illustrates a sequence of events that occurs during smoltification in Atlantic salmon (*Salmo salar*) leading to hypo-osmoregulatory development. Here three groups of Atlantic salmon are presented (anadromous control, parr in February (A) and smolt in May (B) reared under simulated natural photoperiod (SNP); juvenile landlocked salmon in May (C) reared under SNP; and anadromous juveniles in May reared under continuous light (D)) to demonstrate the importance of development of the light-brain-pituitary axis (LBP) early in smoltification on the downstream endocrine output, gill gene expression and hypo-osmoregulatory capacity. The degree of LBP activation is reflected through all downstream processes including morphological and physiological development, here represented by Na^+,K^+-ATPase (NKA) activity (Ebbesson *et al.,* 2007; Nilsen *et al.,* 2007; Stefansson *et al.,* 2007; Ebbesson *et al.,* unpublished data).

years to become manifested under the poorer growth conditions of Northern populations (Heggenes & Metcalfe, 1991; Skilbrei, 1991; Nicieza *et al.*, 1994). Under the exceptionally favourable growth conditions (temperature, photoperiod, food availability etc.) in Atlantic salmon culture, bimodality is normally observed in 0+ juveniles. Much of what we know about bimodal growth comes from laboratory studies, under natural conditions factors such as overlapping year and size-classes, migration and predation may obscure or override an underlying bimodal growth pattern.

Bimodality is the result of differential growth responses within a cohort of juvenile Atlantic salmon, where potential smolts of the upper mode (UM) continue to feed and maintain a relatively high growth rate through the autumn and winter (Metcalfe *et al.*, 1988), while the lower mode (LM) fish cease growth and development. Whether or not to continue growth through winter is a response based on an individual threshold of growth rate and/or surplus energy by summer or early autumn (Thorpe, 1989). Although the physiological mechanisms for size/energy-related developmental phenomena in fish are not fully understood (see below), their relationship to body size is fairly well described. When juvenile Atlantic salmon reach the critical size threshold, approximately 7.5-8.5 cm fork length in autumn among most European stocks, growth rate increases transiently during the 'fast growing phase' (Skilbrei, 1988; Skilbrei *et al.*, 1997). Laboratory studies have demonstrated that specific growth rate during this phase may increase to levels 4-5 times the typical growth rate of salmon parr of this size range, and length growth of as much as 1 mm day^{-1} has been recorded (Benfey *et al.*, 1994). In contrast, the LM fish enter a phase of anorexia during winter (Metcalfe *et al.*, 1986; Metcalfe & Thorpe, 1992), with growth resuming in spring when conditions again become favourable. The LM fish can then enter the UM or remain as LM parr based on their size the following summer. These different life-history strategies are gradually manifested as the UM fish smoltify during the spring, whereas the smaller LM fish delay smoltification and migration for at least one more year. A bimodal length distribution develops even among full-siblings, influenced both by genetics (Stefansson *et al.*, 1990; Nicieza *et al.*, 1994) and environmental conditions (Stefansson *et al.*, 1989; Skilbrei *et al.*, 1997) including food supply to the individual fish.

The underlying mechanisms that control the size-related development of smolts are still largely unknown. It has been proposed that they involve neuroendocrine changes in the brain (McCormick *et al.*,1998, see also Neuroendocrine development below), and studies have shown plasma thyroxine (T$_4$) levels of UM fish to be several fold higher than those of LM fish during winter (Kristinsson *et al.*, 1985), implicating the involvement of the brain-pituitary-thyroid axis. In a recent study, McCormick *et al.* (2007) noted significant differences in the endocrine status of LM (parr) and UM (pre-smolt) Atlantic salmon throughout smolt development. Plasma levels of GH (secreted by somatotropes in the adenohypophysis) of UM fish increased steadily through spring, whereas plasma GH levels of parr remained low and did not respond to increased daylength. Plasma IGF-I levels were substantially lower in LM compared to UM in January and remained below levels of pre-smolts and smolts throughout spring. Similarly, plasma cortisol, which is secreted by the interrenal cells of the head kidney in response to

adrenocorticotropic hormone (ACTH) from the pituitary, was low in LM fish, whereas cortisol levels in UM fish increased through late winter and spring. In line with the findings of Kristinsson *et al.* (1985), plasma thyroid hormones (T_4 and T_3) were generally lower in LM compared with UM fish. The key importance of a functional light-pituitary axis (resulting in elevated secretion of GH) for the initiation of the smoltification process is further demonstrated in a recent study in which juvenile Atlantic salmon were exposed to increased daylength at different body sizes. At 5 cm fork length, no fish responded to increased daylength. At 8 cm, however, fish destined to smoltify responded by a significant elevation of GH levels, while parr <8 cm did not (Nordgarden *et al.*, 2007). The differential activation of these endocrine axes during winter and spring points to fundamental physiological differences between parr and smolt in response to increased daylength, and also emphasises the importance of photoperiod as a is critical factor underlying smolt development. These differences may be explained by divergent development of the light-brain-pituitary axis (LBP; Ebbesson *et al.*, 2003; 2007) resulting in greater photoperiod response of smolt, though the precise pathways involved have not been elucidated (see Neuroendocrine development below).

Key role of growth hormone

Concurrent with the increase in hypo-osmoregulatory ability, other changes occur which are related to growth and metabolism. There is evidence that metabolism of proteins, lipids and carbohydrates is altered, which may relate both to the increased metabolic demands of smoltification and changes in availability and composition of marine prey organisms. Numerous studies have shown that GH has a strong growth-promoting action in salmonids (reviewed by McLean & Donaldson, 1993; Björnsson, 1997). This effect is either direct, or indirect through the subsequent GH induced action of IGF-I (reviewed by Björnsson, 1997, Björnsson *et al.*, 2002; Moriyama *et al.*, 1994; Moriyama, 1995). The growth-promoting effects of GH concern both weight (muscle) growth and length (skeletal) growth. The smoltification-associated changes in growth pattern, which typically includes faster length than weight growth and consequent reduction in condition factor, are fully consistent of the growth-promoting effect of GH in salmonids, and the elevated plasma GH levels during smoltification. Thus, GH treatment of juvenile salmonids usually stimulates length growth more that weight growth, resulting in leaner fish (Higgs *et al.*, 1975, Johnsson & Björnsson, 1994; Neregård *et al.*, 2007). Recently, Wargelius *et al.* (2005) demonstrated that in Atlantic salmon, a peak in GH-receptor expression in vertebral bone tissue is associated with growth activation, while an upregulation of the IGF-I-receptor in vertebral bone appears to be related to increased bone density.

The stimulating effect of GH on body growth is further associated with increased food intake (Markert *et al.*, 1977; Johnsson & Björnsson, 1994) and increased feed conversion efficiency (Markert *et al.*, 1977; Gill *et al.*, 1985; Garber *et al.*, 1995). The effects of GH on food intake are not fully explained, but higher GH levels are associated with behavioural changes such as increased swimming activity, aggression and

dominance, as well as decreased predator-avoidance (Johnsson *et al.*, 1996a; b; 1999; 2000; Jönsson *et al.*, 1996; 1998), consistent with increased opportunity to obtain food. The increased growth of GH-treated salmon parr further suggests an elevated foraging activity within the territory (Martin-Smith *et al.*, 2004). Recent studies indicate that in part, the behavioural effects of GH in salmonids may be direct, at the level of the central nervous system (Jönsson *et al.*, 2003; Johansson *et al.*, 2004; 2005).

In terms of tissue metabolism, the two major effects of GH in salmonids appear to be the stimulation of lipid mobilisation and protein accretion (Björnsson, 1997), but few studies have been carried out in relation to smoltification. The anabolic effects of GH on protein metabolism in salmonids have been studied most closely in rainbow trout (Foster *et al.*, 1991), where GH is found primarily to stimulate protein synthesis, and to a lesser extent to decrease protein degradation. In Atlantic salmon, standard and active metabolic rates are about 50% higher in smolts than in parr. The reasons for this are suggested to be due to elevated respiratory enzyme activity and mitochondrial proliferation (Maxime *et al.*, 1989) and to the metabolic demands of development and differentiation (McCormick & Saunders, 1987). Oxygen consumption is also seen to increase in smolts, and this increase is stimulated by several hormones, including GH (see above; Seddiki *et al.*, 1996). However, the increased metabolism/oxygen consumption may partly be due to increased physical activity such as seen following GH treatment (Herbert *et al.*, 2001). Smolts typically show higher cardiosomatic index (CSI) than parr (Poupa *et al.*, 1974; Leonard & McCormick, 2001). Heart growth is accelerated (relative to body growth) during smoltification, probably as an adaptation to the increased cardiac output required for the pelagic schooling of the marine salmonids. Another developmental event which is worth noting in this context is the changes in the oxygen-binding proteins (haemoglobins) of the blood. There is conflicting evidence whether these changes are specific to smoltification or whether they are more related to the continuous increase in size and hence not strictly an aspect of smoltification (Hoar, 1988; Fyhn *et al.*, 1991). An increasing complexity of haemoglobins has been described in parr, smolts and post-smolts of *Oncorhynchus* and *Salmo* species, and is generally considered to be an adaptation to the differences in availability of oxygen between fresh water and seawater and the changes in behaviour, feeding and growth.

There is also some evidence of a reorganisation of amino acid distribution during smoltification; however, previous studies have reported no net change in the total amino acid content of smolts (Fontaine & Marchelidon, 1971). It has been suggested that this reorganisation is linked to guanine and hypoxanthine deposition in the skin and scales (smolt silvering, Fontaine & Marchelidon, 1971) as well as possible changes in cellular osmoregulatory gradients (McCormick & Saunders, 1987). There is also evidence of an increased activity of respiratory enzymes in the gill (Langdon & Thorpe, 1985; Chernitsky, 1986; McCormick & Saunders, 1987) and liver (Blake *et al.*, 1984) of Atlantic salmon smolts. From a growth and energetics perspective, smolts appear to prioritise protein deposition and growth of muscle and skeleton, at the expense of reduced lipid and carbohydrate stores (see Stefansson *et al.*, 2003a and discussion

below). Studies under controlled conditions demonstrate an increase in growth rate following seawater transfer, despite no significant change in temperature or feeding (Handeland & Stefansson, 2001; 2002; Arnesen *et al.*, 2003), suggesting an influence of salinity itself on growth performance. The physiological basis of this growth increase may be related to the increase in GH observed as a response to salinity in salmonids (Björnsson, 1997; Björnsson *et al.*, 2000; Arnesen *et al.*, 2003; Handeland *et al.*, 2003).

Smolt development is generally accompanied by decreases in lipid and glycogen reserves in liver and muscle, even when food is present in excess (Wendt & Saunders, 1973; Sheridan, 1989). In coho salmon the reduction in liver glycogen during smoltification is caused by a combination of decreased glycogen synthesis and increased glycogenolysis (Sheridan *et al.*, 1985). Further studies of the biochemical basis for the changes in energy status during smoltification in Atlantic salmon have revealed both a seasonal, environmental and developmental tissue-specific regulation of several key metabolic enzymes (Leonard & McCormick, 2001). Smolts consistently showed lower liver lactate dehydrogenase (LDH), suggesting a decreased reliance on lactate–pyruvate conversion in line with findings by Plisetskaya *et al.* (1994) and Ji *et al.* (1996) who found low concentrations of blood glucose and liver glycogen due to decreased gluconeogenesis. Of potentially particular significance were changes in white muscle phosphofructokinase (PFK), which increased in both wild and hatchery-reared smolts but not in hatchery-reared parr. The same metabolic phenomenon was observed in white muscle LDH which showed an earlier increase in hatchery-reared than wild smolts and wild parr. This suggests that both habitat and developmental stage may influence metabolic parameters (Leonard & McCormick, 2001). Recent evidence from anadromous Arctic charr (*Salvelinus alpinus*) demonstrates an increase in activity of several hepatic enzymes concurrent with a reduction in liver glucose and glycogen (Aas-Hansen *et al.*, 2005), supporting the concept of smolts as glucose deficient. The results of Stefansson *et al.* (2003a, see below) support this view, and demonstrate that smolts have low energy reserves during the early marine phase.

Lipid metabolism is altered during smoltification resulting in reduced body and muscle lipid (Saunders & Henderson, 1970; 1978), often observed as a reduction in condition factor. Studies of chinook salmon (*Oncorhynchus tshawytscha*, Cowley *et al.*, 1994) and coho salmon (Sheridan *et al.*, 1985; Sheridan, 1986) suggest that this depletion of lipid stores is caused by increased lipolysis and decreased fatty acid synthesis. In coho salmon parr, Sheridan (1986; 1994) found GH to increase triacylglycerol (TAG) lipase activity, whereas GH treatment of Atlantic salmon smolts failed to raise plasma FA levels (McKeown *et al.*, 1975) or hepatic lipid mobilisation (Sheridan, 1986). Possibly, elevated endogenous GH levels during smoltification have already increased the rate of lipolysis to a level at which GH treatment is ineffective. The increased activity of several lipases during smoltification (Sheridan, 1994) suggests that the lipid depletion is not a function of reduced feeding, but rather an integral part of the changes preparing the smolt for migration and marine life. Changes in specific lipid classes may also be an important part of smolt development. Li & Yamada (1992) observed a proportional

decrease of TAG and an increase of phospholipid (PL) in the gut and gill of masu salmon (*Oncorhynchus masou*) smolts in seawater and concluded that the increased proportion of (n-3) polyunsaturated fatty acids (PUFA), particularly in polar lipids of osmoregulatory organs (gut, gill) was critical for seawater adaptation.

As for other preparatory changes associated with smoltification, the changes in lipid metabolism are reversible, and qualitative and quantitative reversions to a pre-smolt stage are observed if smolts are maintained in fresh water. In a study of tissue lipid composition of smolts, seawater post-smolts and desmoltified masu salmon, Li & Yamada (1992) concluded that lipid contents of muscle, liver, gut and gills of desmolts increase compared with smolt levels, and further, that the proportion of TAG increases while PL decreases in tissue lipids of desmolts compared with smolts. As would be expected based on the discussions above, tissue lipids of desmolts contain more mono-unsaturated fatty acids and saturated fatty acids and less PUFA, especially (n-3) PUFA such as 22:6(n-3), than those of smolts in fresh water and post-smolts in seawater.

Freshwater prey organisms are rich in 18:2(n-6) and 18:3(n-3) fatty acids in contrast with the marine diet which is rich in the longer chain highly unsaturated fatty acids (HUFA) such as 20:5(n-3) and 22:6(n-3), hence marine fish differ from freshwater fish in their fatty acid composition, and are generally characterised by higher proportions of PUFA (Sargent *et al.*, 1999). It is important to note that smolts initiate these preparatory changes in both fatty acid metabolism and tissue fatty acid composition while still in fresh water. This includes an increase in fatty acid elongase and desaturase activity of isolated hepatocytes as well as increases in the ratio of C20 and C22: C18 and n-3: n-6 fatty acids in salmonid tissue (Tocher *et al.*, 2000; 2003). HUFA biosynthesis in Atlantic salmon reaches a transient peak activity at the time of completion of smoltification and seawater transfer (Tocher *et al.*, 2003; Zheng *et al.*, 2004). Consistent with this, the gene expression of ?6 desaturase, the rate-limiting step in the HUFA biosynthetic pathway, is highest around the point of seawater transfer.

In addition to the changes occurring in the main lipid stores (muscle, mesenteric fat), changes have been described in other tissues, such as gills, during the early seawater phase (Handeland *et al.*, 2000). This occurs even without a change in diet, suggesting that these changes in lipid composition are related to functional changes in the gill tissue (e.g., gas-transfer, ion regulation, acid-base balance). An important smolt-related feature of gill lipid metabolism is the transient reduction in prostaglandin F (PGF) production at seawater transfer (Bell *et al.*, 1997; Tocher *et al.*, 2003), with peaks of activity before and after transfer (Bell *et al.*, 1997). Whether this is a developmental (Bell *et al.*, 1997) and/or seasonal (Tocher *et al.*, 2003) phenomenon remains to be fully elucidated. Prostaglandins are known to modulate adaptation to salinity alteration in ectothermic animals (Mustafa & Srivastava, 1989). Gill prostaglandin F2α (PGF2α) production increases in Atlantic salmon post-smolts with increased gill polar lipid arachidonic acid (ARA) and decreases eichosapentinoic acid, EPA/ARA ratio (Bell *et al.*, 1997a; Tocher *et al.*, 2000; Bell & Sargent, 2003). In addition, fish with higher ARA levels in the gill PL tend to adapt better to seawater, implicating the importance of feed composition in

stimulating preparatory changes in smolts and improving their capacity to respond appropriately to salinity change (reviewed by Bell & Sargent, 2003). Changes in PGF production may also have consequences for disease susceptibility of smolts, which are characterised by changes in serum proteins, immunoglobulin M (IgM) levels and leucocyte populations, which may affect cellular and humoral immunity (Maule *et al.*, 1989; Melingen & Wergeland, 2000; Melingen *et al.*, 2002; see also Zapata & Cortés, this volume).

The results of Stefansson *et al.* (2003a) on wild migrating smolts in river and coastal waters support the models outlined above, and further demonstrate that wild smolts have low energy reserves also during the early marine phase. Lipid and glycogen content decreased towards depletion from the river, through the fjord and into the ocean. There was, however, no significant change in protein content. These results confirm that smolts are naturally 'energy deficient' during downstream migration, and suggest that post-smolts also mobilise energy reserves during their early marine phase. Plasma GH levels increased transiently during passage through the estuary and fjord, with lower levels observed in post-smolts caught off-shore, i.e. in fish which were feeding on marine prey and had adapted to the marine environment. Taken together, these findings suggest that post-smolts maintain a positive protein balance, and that there is a high scope for growth during this period, at the expense of energy stores. This model is consistent with increasing RNA:DNA ratios in wild post-smolts from estuary through fjords and into the ocean (Haugland, Stefansson, Bjørn, Finstad & Hvidsten, unpublished data).

Ion/Osmoregulation

Perhaps the single most critical element of smoltification is the development of hypo-osmoregulatory ability (Fig. 1). Prior to smolt development parr have relatively poor seawater tolerance; and direct transfer of parr to seawater results in high levels of ionic and osmotic perturbation, increased mortality and reduced growth. These responses are only partly due to the size difference between parr and smolt, since fish large enough to become smolts but prevented from doing so by photoperiod manipulation have reduced hypo-osmoregulatory ability and growth in seawater (McCormick *et al.*, 1987, Stefansson *et al.*, 1991; 2007). The higher salinity tolerance of smolts permits rapid movements through estuaries and into coastal waters and reduces predation (Handeland *et al.*, 1996).

All teleosts maintain a relatively narrow range of osmotic pressure in their extracellular fluid, generally 260-360 mOsm kg^{-1}. Where salmonids are found, fresh water is normally 1–10 mOsm kg^{-1}, and fish counteract the passive gain of water and loss of ions by having the kidney produce an abundant and dilute urine, and by actively taking up salts across the gill. Salts may also be absorbed from food in the intestine. In seawater ($\sim$1000 mOsm kg^{-1}), fish reverse the passive loss of water and gain of ions by drinking seawater, absorbing water and monovalent ions across the gut and excreting Na$^+$ and Cl$^-$ over the gills and divalent ions by the gut and kidney.

The role of chloride cells in ion transport

In juvenile and adult teleosts, the gill is a critical site for ion regulation. It is also the site for a wide range of other critical physiological functions for maintaining homeostasis. It is the site for gas exchange, ammonia excretion, and plays a critical role in acid/base balance (see Brauner, this volume; Pelster, this volume; Terjesen, this volume). In the context of ion regulation and acid base balance, the mitochondrion-rich chloride cells (CC, Fig. 2) play a key role in uptake and excretion of Na^+, Cl^-, HCO_3^- and H^+. A detailed discussion of ion regulation in fish larvae can be found in Kaneko (this volume), however, we have included a brief summary, based on McCormick (2001), Marshall (2002), Evans *et al.* (2005), of current models for transport of monovalent ions.
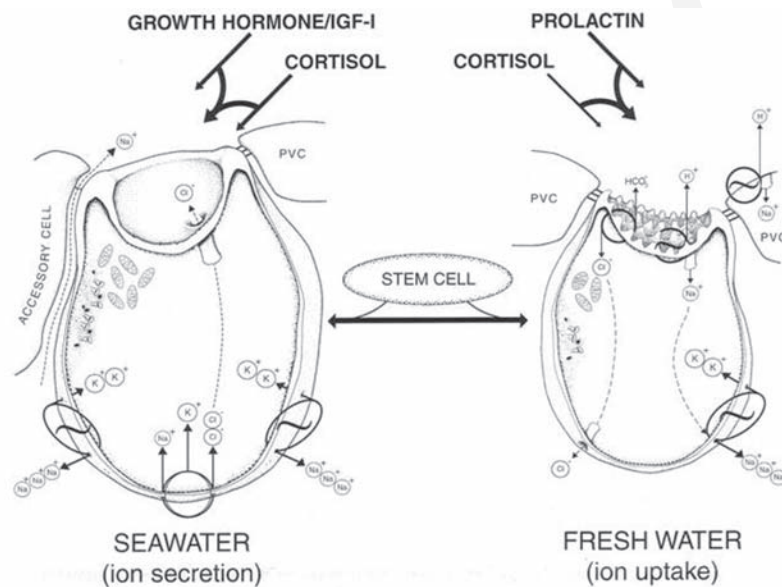

Fig. 2 Morphology and transport mechanisms of gill chloride cells in seawater and fresh water. See text for details of transport mechanisms. Chloride cells are characterised by numerous mitochondria and an extensive tubular system that is continuous with the basolateral membrane. In seawater, chloride cells are generally larger and contain a deep apical crypt, whereas in freshwater the apical surface is broad and contains numerous microvilli. In some species, such as tilapia the H1-ATPase and apical sodium channel may be present in pavement cells rather than chloride cells. Recent evidence suggests that individual chloride cells can move between these two morphological states (Hiroi *et al.*, 1999), and also arise from undifferentiated stem cells (Wong & Chan, 1999). Growth hormone and cortisol can individually promote the differentiation of the seawater chloride cell, and also interact positively to control epithelial transport capacity. Prolactin inhibits the formation of seawater chloride cells and promotes the development of fresh water chloride cells. Cortisol also promotes acclimation to fresh water by maintaining ion transporters and chloride cells, and by interacting to some degree with prolactin. PVC = pavement cell. From McCormick (2001).

Mechanisms of Ion uptake in freshwater

To remain hyper-osmotic in fresh water, fish take up ions (Na^+, Cl^-, Ca^{2+}) from the water by active and secondary active transport mechanisms to compensate for passive ion loss, and produce large volumes of dilute urine to balance the osmotic gain of water. Ion uptake across the gill is an active area of research, and there remains some uncertainty as to the mechanisms involved among teleost groups and in different fresh water environments. Current consensus suggests that Na^+ moves across the apical membrane either through exchange with H^+ (NHE) or through an epithelial Na^+-channel (ENaC) energised by H^+-ATPase. Na^+ is then moved across the basolateral membrane by NKA. Apical Cl^-/HCO_3^- exchange mechanisms have been found in the apical membrane of CCs, implicating HCO_3^- excretion as a driving force for Cl^- uptake. The channels or transporters involved in movement of Cl^- across the basolateral membrane and into the blood have yet to be characterised, but probably involve the CFTR channel.

Mechanisms of Ion excretion in seawater

Marine teleosts drink seawater to replace osmotic water loss, and excrete excess Na^+ and Cl^- across the gills through CCs. Basolateral NKA provides both chemical and electrical gradients for Na^+ and Cl^- secretion. By transporting $3\,Na^+$ for $2\,K^+$, NKA generates an intracellular electronegative gradient (approx. $-100\,mV$) low in Na^+, whereas the blood becomes high in Na^+ and positive. The large Na^+ gradient created by NKA allows a basolateral NKCC to shuttle Cl^- into the CC. Cl^- can then leave the highly negative CC on a downhill electrical gradient through and an apically located CFTR anion channel. Na^+ exits through a paracellular pathway between the CC and its accessory cells on a downhill electrical gradient (also created by NKA). The mechanistic basis of the paracellular Na^+ pathway has yet to be investigated in fish.

Development of salinity tolerance: cellular and biochemical basis

The development of salinity tolerance is functionally linked to increases in transport proteins and the number and size of CC. Seawater CCs are characterised by an extensive tubular system, continuous with the basolateral membrane, creating a large surface area for transport proteins, and the presence of a large apical crypt (McCormick, 2001; Marshall, 2002, Fig. 2). The appearance of these morphological features of seawater CC have been shown to develop in spring in smolts while still in fresh water, and to be absent in parr (Payan *et al.*, 1984; Pisam *et al.*, 1988; Lubin *et al.*, 1989;). Seawater CC are also larger than those in fresh water, and numerous studies have shown that the number and size of CCs increase during smoltification but subsequently decrease (along with salinity tolerance) if fish remain in fresh water after smoltification (McCormick, 2001).

Gill NKA activity and protein abundance has been shown to increase during the spring and to be much higher in smolts compared to parr. There is a strong genetic

component to NKA expression that can explain performance difference between strains (Nilsen *et al.*, 2003, Mackie *et al.*, 2005). NKA consists of three subunits (α, β and γ), and several isoforms of each of these subunits have been found in vertebrates. Richards *et al.* (2003) found that the α1a subunit mRNA levels decrease and the α1b subunit mRNA levels increase during seawater acclimation of rainbow trout. In Atlantic salmon, α1a mRNA levels decrease and α1b mRNA levels increase during smolt development (Nilsen *et al.*, 2007). These data suggest a possible differential role of these two isoforms in fresh water and seawater, though to date the protein levels, localisation and physiological significance of these isoforms have yet to be characterised.

Other ion transporters

NKCC has been localised to the basolateral membrane of CCs (Fig. 2), and appears to be regulated by the same developmental program of chloride cell differentiation as NKA. The abundance of NKCC protein shows a clear increase during smoltification of Atlantic salmon (Pelis *et al.*, 2001). Similarly, the number of NKCC-immunoreactive chloride cells also increases during smolt development and decreases coincident with loss of salinity tolerance. Photoperiod manipulation results in altered timing of NKCC levels, and correlates with coincident changes in NKA and salinity tolerance (Stefansson *et al.*, 2007). Nilsen *et al.* (2007) found that NKCC abundance and mRNA levels were higher in anadromous relative to land-locked populations of Atlantic salmon.

Atlantic salmon express at least two isoforms of the CFTR anion channel, CFTR I and II (Singer *et al.*, 2002). CFTR I and II mRNA levels increase in Atlantic salmon smolts in response to seawater, though the latter only transiently (Singer *et al.*, 2002). Increased CFTR mRNA levels have also been found during smoltification, and are higher in anadromous relative to landlocked strains of Atlantic salmon during spring (Nilsen *et al.*, 2007). Gill CFTR I mRNA levels also decrease during disruption of normal smoltification by exposure to acid/aluminum (Monette, McCormick and Yada, unpublished data) or estrogenic compounds (Lerner, McCormick and Yada, unpublished data).

The role of the intestine in hydro-mineral balance

The increase in ionic concentration, prey availability and composition, and scope for growth associated with marine life require alterations in uptake and transport mechanisms in the intestine. A functional regionalisation of the intestine appears to be part of this preparatory adaptation for life in the marine environment. In fresh water, drinking rate is low and ions are absorbed from dietary sources, in addition to an active uptake across the gills, to replace losses to the hypotonic medium. During smoltification and seawater acclimation, changes take place which are characteristic of marine teleosts. The fluid transport rate (J_v) of the anterior and mid-sections of the intestine decrease from parr levels of 15-20 μl cm^{-2} h^{-1} to below 10 μl cm^{-2} h^{-1}, while the posterior sections show a distinct increase from typical parr levels of 10-15 μl cm^{-2} h^{-1} to 20-25 μl cm^{-2} h^{-1} in smolts, causing an overall 2-3 fold increase in intestinal fluid absorption in

smolts (Collie & Bern, 1982; Veillette *et al.*, 1993; 1995, Sundell *et al.*, 2003). This increase in fluid transport is functionally linked to increased NKA activity (Veilette *et al.*, 1993) and epithelial paracellular permeability (Sundell *et al.*, 2003). Acclimation to SW involves increased drinking rate (Usher *et al.*, 1988; Nielsen *et al.*, 1999), a further increase in intestinal NKA activity, generally concomitant with a further increase in fluid absorption of the posterior intestine beyond the freshwater preparatory levels (Veillette *et al.*, 1993), and a shift in intestinal water uptake from a paracellular to a transcellular pathway (Sundell *et al.*, 2003). The discovery that aquaporins are recruited in the intestine of marine adapted eels (Lignot *et al.*, 2002), suggests that a similar mechanism of transcellular water uptake may be present in salmonids. Cortisol appears to be a major endocrine factor controlling these changes in intestinal fluid transport (Veillette *et al.*, 1995, 2004; Sundell *et al.*, 2003). In addition to their role in nutrient uptake, recent evidence also indicates that the pyloric caeca are important sites for hydro-mineral balance in smolts (Nielsen *et al.*, 1999; Veilette & Young, 2004), since they show similar changes in NKA activity and responsiveness to cortisol to that of the posterior intestine.

NEUROENDOCRINE CONTROL

The endocrine system and how it regulates smoltification processes has been studied for more than six decades and has been the subject of numerous reviews on growth, osmoregulation, imprinting, metabolism and its regulation (Hoar, 1988; McCormick *et al.*, 1998). What has been less studied and is emerging now as a key element in the smoltification process is the development of the regulator of the endocrine system, the brain (Ebbesson *et al.*, 2003; Ebbesson *et al.*, 2007). Here we briefly present the LBP and illustrate how structural changes in the LBP occurs prior to the characteristic endocrine surges, indicating that this change in the LBP may make the neuroendocrine system more photosensitive. Further, we discuss brain changes during smoltification and where possible relate their individual roles to the smoltification processes.

Light-brain-pituitary Axis and Endocrine Influence

Environmental information is transduced through sensory processes to neuroendocrine responses in the brain, that subsequently control pituitary function and ultimately physiological responses through several endocrine axes. The LBP is the pathway by which photoperiod information is conveyed to the endocrine system. Light is detected by the retina and pineal organ, which transmits this information through neurons projecting to central brain regions, one of which is the preoptic area (POA). Here the information is integrated with other neural inputs and relayed to the pituitary, regulating hormone release into circulation (Holmqvist *et al.,* 1992). Of the wide range of hormones known to be involved in smoltification, cortisol, GH and TH are key regulators that display sequential increases in circulating levels that promote their transformational roles (Hoar, 1988; McCormick *et al.*, 1998; Björnsson *et al.*, 2002). Thyroid stimulating
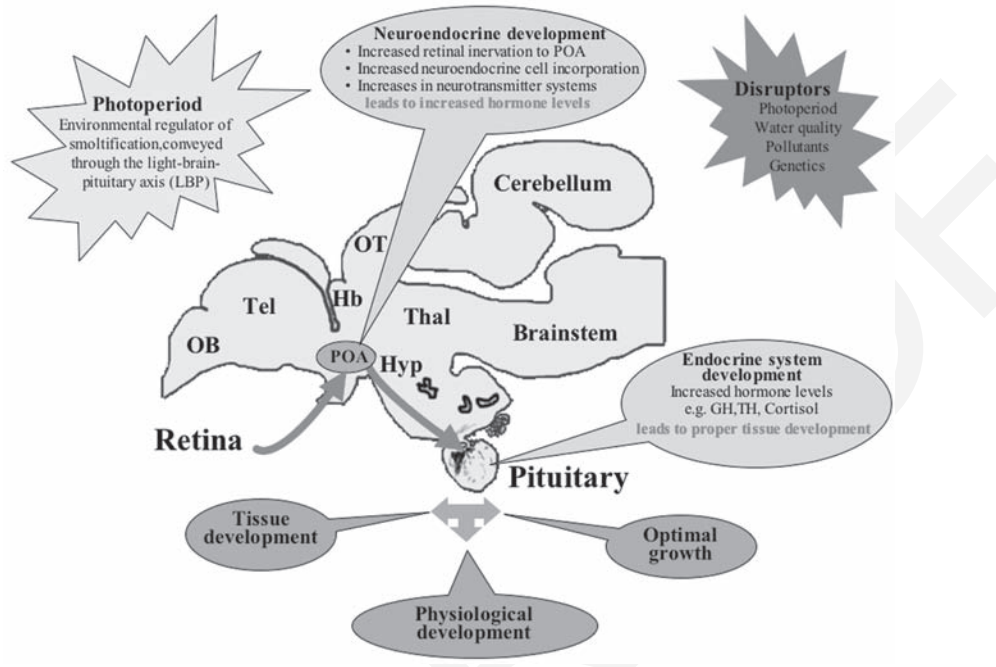
Fig. 3 An overview of the light-brain-pituitary axis that illustrates the role of brain changes during smoltification on endocrine and physiological development. Abbreviations: OB, olfactory bulb; Tel, telencephalon; Hb, habenula; POA, preoptic area; OT, optic tectum; Hyp, hypothalamus; Thal, thalamus; GH, growth hormone; TH, thyroid hormone.

hormone (TSH) is released from the pituitary and stimulates the production and release of TH. GH is released from the pituitary into the circulation and ACTH regulates the production and release of cortisol from the head kidney (Holmqvist *et al.*, 1994; Holmqvist & Ekström, 1995; Baker *et al.*, 1996; Holloway & Leatherland, 1998; Ágústsson *et al.*, 2000). The releases of TH, GH and cortisol are directly or indirectly regulated by complex negative and positive feedback systems, including the actions of DA, GnRH, serotonin (5-HT), corticotropin releasing factor (CRF), thyrotropin releasing hormone (TRH), opiate peptides, or arginine vasotocin (AVT, Eales, 1995; Baker *et al.*, 1996; Björnsson *et al.*, 2002; Canosa *et al.*, 2007).

Smolt-related increases in circulating hormones have shown that they often act synergistically to affect hypo-osmoregulatory capacity, morphological changes (silvering of scales and skin), metabolic changes and schooling behaviour. For example, cortisol, in addition to its overall role in energy metabolism, regulation of protein turnover and lipid mobilisation, has important roles in the proliferation and differentiation of gill CC, gill NKA, NKCC and salinity tolerance. These functions are mediated through the interactions of cortisol with GH, IGF-I and corticosteroid receptors (Björnsson *et al.*, 1995; Björnsson, 1997; Seidelin *et al.*, 1999; McCormick, 2001). THs have also been

shown to be essential in many smoltification related processes including silvering, olfactory imprinting, behaviour, and changes in visual sensitivity (Morin *et al.*, 1995; Hutchison & Iwata, 1998; Specker *et al.*, 2000; Power *et al.*, 2001; Lema & Nevitt, 2004; Allison *et al.*, 2006). Despite these studies, mechanisms of TH action during smoltification are still largely unresolved, most likely due to the complex TH regulation (Eales *et al.*, 1993; Morin *et al.*, 1993; Eales, 1995), changes in the system (Richardson *et al.*, 2005) and their often synergistic role in many of these key processes. TH are known to act synergistically with a range of other hormones, including cortisol, GH and prolactin (PRL), likely through modulation of receptor characteristics, as is shown for the corticosteroid receptors (McCormick, 2001). The changes in hormone levels have been studied extensively, yet only recently has the development of the neuroendocrine system become a focus and revealed its critical role in opening a 'gate' that increases neuroendocrine responsiveness to environmental photic inputs (see Fig. 6; Ebbesson *et al.*, 2003; 2007; McCormick *et al.*, 2007).

Neuroendocrine Development

Our recent data emphasise that the structural reorganisation of specific brain regions occurs prior to the major hormone surges that regulate the smoltification processes (Ebbesson *et al.*, 2003), while changes in neurotransmitter systems occur throughout smoltification regulating neuroendocrine and behavioural processes (Ebbesson *et al.*, 1996a; b; Morin *et al.*, 1997). Neural tract tracing has shown that some of these changes involve increased innervation of retinal and pineal fibres into POA and other brain regions (Holmqvist *et al.*, 1994; Ebbesson *et al.*, 2003; 2007), and a specific period of cell differentiation, as shown by transient growth-associated protein-43 (GAP-43) immunoreactive cells and fibres (Ebbesson, 2000; Ebbesson *et al.*, 2003). These structural reorganisations of the brain occur prior to the major increases in circulating TH, GH and cortisol levels, pivotal hormones in the physiological processes of smoltification (Ebbesson *et al.*, 2003). These data support the model that increased retinal innervation of the POA facilitates the increased endocrine response to changes in photoperiod.

In addition to structural reorganisation during smoltification, there are changes in neurotransmitter systems including the transient appearance of serotonergic neurons in the habenula and the lateral preoptic area (Ebbesson *et al.*, 1992) and the appearance of GnRH in the basal forebrain and midbrain of chum salmon (Lewis *et al.*, 1992; Parhar & Iwata, 1996). There are also sequential changes of certain neurotransmitter levels, e.g. GnRH, 5-HT, DA and norepinephrine (Lewis *et al.*, 1992; Ebbesson *et al.*, 1996b) in specific regions of the brain. Some of these systems are affected by thyroid hormones (Morin *et al.*, 1997) and environmental/rearing conditions (Ebbesson *et al.*, 1994). There are also temporal changes in opiate receptors (Ebbesson *et al.*, 1996a) and TH receptors (Kudo *et al.*, 1994) in specific brain regions. It is hypothesised that thyroidal status in the brain affects monodeiodinase activities (Eales *et al.*, 1993; Morin *et al.*, 1993) that appears to be related to olfactory sensitivity (Morin *et al.*, 1994). Additional changes of the LBP axis include changes in retinal UV photosensitivity and

visually-evoked electrophysiological responses in the telencephalon and torus semicircularis (Hofmann & Meyer 1993; Browman & Hawryshyn, 1994). Such data suggest that neural development during critical periods of development precede the physiological changes that direct life histories and fitness. At this point our understanding of larval and juvenile brain development in salmon is well underway, but the effects of undesirable environmental influences on brain development and smoltification during these critical periods in salmon are only partly known.

In salmon, as in other vertebrates, the life cycle includes specific critical periods of neural development (e.g., early development, smoltification, and sexual maturation). Disruption of the developmental program profoundly impacts the future outcome. These life history stages are finely tuned and sensitive to environmental stimuli that synchronise the proper timing of the development processes to maximise survival (McCormick *et al.,* 1998). Genetic specification determines much of the basic structure and function of the nervous system, however, the environment to a great extent affects the timing of development of neural pathways and functions. These developmental processes are guided by evolutionary and ontogenetic principles, partly explained by 'the parcellation theory' (Ebbesson, 1980). Thus, once connections are established, the survival of the new connections are dependent on the subsequent environmental stimuli, moulding the brain, by maintaining stimulated synapses, and losing unused connections to establish functional circuits in order for the animal to
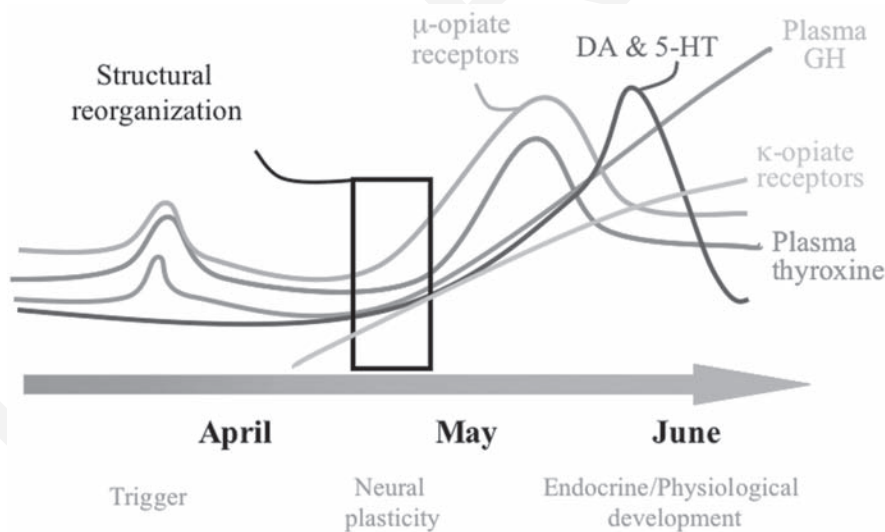

Fig. 4 Schematic diagram showing temporal changes in brain and endocrine systems during salmon smoltification. Early structural brain changes (box; e.g. retinal tract tracing) are followed by changes in neurotransmitter systems (e.g. opiate receptors, dopamine, DA and serotonin, 5-HT) and surges in circulating hormone levels (e.g thyroxine, growth hormone, GH; Ebbesson *et al.*, 1992; 1996a; b; 2000; 2003).

progress into the next stage of life. Indeed, the tailoring of neuronal circuits to the requirements of each individual may be considered to be the basis of critical periods and in salmon can lead to a variety of life strategies. Recent evidence suggests the importance of normal brain development during smoltification to attain the necessary endocrine and physiological changes (Ebbesson *et al.*, 2007).

PHYSIOLOGICAL BASIS FOR MORPHOLOGICAL CHANGES

Increased silvering is probably the best known external characteristic of salmonid smoltification (Fig. 1). The overall silvery colour characteristic to the smolt is caused by the deposition of purines (guanine and hypoxanthine) in the scales, scale layer and *stratum argentum* layer of the skin (Johnston & Eales, 1967; Staley & Ewing, 1992). Both layers are thicker in smolts than in parr. Consequently, the characteristic parr-marks ('finger marks') are obscured by layers of silvery skin and scales, however, they remain in the deeper skin layers and may become visible if the outer skin layers are eroded. Concurrent with the increased silvering, the juvenile salmon develops black fin margins, particularly on the dorsal, caudal and pectoral fins, while the fins become lighter, almost translucent. These changes are easily observed when schools of smolts are viewed either in nature or in culture. There is no firm evidence for the adaptive significance of these changes, however, it can be hypothesised that the contrasting fin margin, especially of the caudal fin, contributes to visual signalling within a school of smolts.

The scales of smolts are looser than in parr, and are therefore easily dislodged during handling. It is difficult to propose any adaptive significance of loose scales, rather, this phenomenon should perhaps be viewed as a consequence of the increased growth rate observed in smolts. Evidence from the culture of Atlantic salmon suggests that loose scales are observed during periods of rapid growth, hence it is suggested that this may be related to the turnover of connective tissue associated with growth processes. From a practical point of view, scale losses from handling, netting and transportation of smolts will reduce the barrier towards osmotic disturbances and increase the osmoregulatory challenge associated with seawater entry.

The increased deposition of purines in the body may represent an alternative biochemical route for nitrogen metabolism which serves a dual purpose (Hoar, 1988; see also Terjesen, this volume). Firstly, by permanently storing purines in the body, no further energy consuming enzymatic oxidation to soluble products is required, hence water is not wasted in excreting such compounds. This may be regarded as an integral part of adapting to the hyper-osmotic conditions of seawater. Secondly, purine storage in the skin contributes to the adaptive counter-shading camouflage characteristic of pelagic schooling fish species. The endocrine basis for the changes in protein metabolism which cause the increased silvering is still not fully resolved, however, activation of the TH axis is known to increase purine deposition in teleosts. These changes are generally not as intense as the silvering observed in normal smolts, suggesting synergy with other

hormones. GH has been implicated in stimulating silvering as has been seen in eels (Durif *et al.*, 2005). However, in a recent study using long-term GH-implants in Atlantic salmon parr, the silvering effect was found to be size-dependent rather than GH-dependent (Neregård *et al.,* unpublished data). The importance of these synergies are further supported by the observations that TH are ineffective in inducing the development of black fin margins, rather increased ACTH (but not cortisol) has been shown to cause the darkening of the dorsal surface and fin margins of juvenile Atlantic salmon (Langdon *et al.*, 1984).

ENVIRONMENTAL REGULATION

Photoperiod

From the late 1960s extensive research has been carried out to define the environmental control of smoltification. In several salmonids, including the Atlantic salmon, the most important environmental cue for the synchronisation of these processes is the seasonal changes in photoperiod. Concurrent with these findings, studies have suggested that important physiological changes related to smoltification, e.g. increased growth rate, seawater tolerance, silvering and reduced condition factor may occur independently and in the absence of environmental signals (*zeitgeber*; Eriksson and Lundqvist, 1982). Such endogenously driven circannual rhythms will, however, generally be unsynchronised, as the circannual rhythms are not sufficiently precise in the absence of a zeitgeber. The different aspects of smoltification may occur out of phase and out of step with the time of year. Further, the intensity of such changes is lower than in smolts, and despite a certain degree of silvering and somewhat improved seawater tolerance (Fig. 1, see further below), such incompletely smoltified juveniles often fail to adapt normally to seawater, hence survival and growth may be highly variable and disease outbreaks and mortalities often occur. Signals from the environment are needed to synchronise the processes, and juvenile Atlantic salmon detect and respond to changes in daylength. There is a large literature on the impact of normal and artificial photoperiods on smolt development (see references in Stefansson *et al.*, 2007)

Aspects of the biological clock have been studied in model teleosts such as zebrafish, and evidence points to conserved elements in the organisation of biological time-keeping in teleosts as in other vertebrates. In the context of smoltification, postulated circannual clocks are likely the most important as they translate changes in daylength into a physiological response. In contrast to the circadian system, hardly any information is available on the circannual time-keeping system in lower vertebrates. The current vertebrate model predicts the presence of integrative zones, calendar cells, which translate seasonal and diel changes in melatonin levels into long-term changes in physiology. Although very limited information is available for salmonids, studies of the rainbow trout have shown the expression of *clock*, a key time-keeping gene, in γ-aminobutyric acid (GABA) neurons which also express the melatonin receptor, localised to specific regions of the brain. These regions are suggested to be the sites at which

environmental information is integrated into the biological clock to influence behavioural and endocrine responses (Mazurais *et al.*, 2000).

Growth rate is also significantly influenced by photoperiod, being high in spring and early summer as daylength is increasing, with a subsequent reduction later in summer and autumn as daylength decreases. Growth rate often shows better correlation with photoperiod than with temperature. In Atlantic salmon culture, long photoperiod and continuous light may be used to enhance growth rate of juvenile salmon in freshwater, and is routinely used during first-feeding, and to maintain juvenile growth during autumn and early winter. Correct use of photoperiod manipulation is used to improve survival during first-feeding and to increase subsequent growth rate, number of potential 1+ smolt and smolt size. Extended use of continuous light will, however, interfere with the completion of smoltification, as such unnatural photoperiods through winter and spring desynchronise the smoltification process (Fig. 1, McCormick *et al.*, 1987; Stefansson *et al.*, 1991). Such 'pseudo-smolts' will differ in smolt status at the time of release, and seawater performance will, at best, be variable. Recent evidence on the importance of a natural photoperiod for the completion of smoltification, and the failure of juvenile salmon to develop under constant light conditions demonstrate that smoltification is disrupted at a fundamental level (Ebbesson *et al.*, 2007; Stefansson *et al.*, 2007).

Parr exposed to continuous light, LL, do not develop the same extensive retinal innervation to the preoptic nucleus (NPO, Ebbesson *et al.*, 2007) that is seen in control Atlantic salmon smolts raised under natural or simulated natural photoperiod (Fig. 5, Ebbesson *et al.*, 2003; 2007). These retinal fibres, in part making up the light-brain section of the LBP, terminating on neurohypophysiotropic cells (e.g. CRF and AVT) in the NPO that either directly or indirectly regulate the release of pituitary hormones (e.g. GH, TSH, ACTH) or directly release the neurohormones into the circulation via the pituitary (e.g. AVT and oxytocin; Holmqvist *et al.*, 1994; Ebbesson *et al.*, 2003). Recent data show that, in addition to the lack of increased retinal innervation in the LL parr, they also do not increase the number of CRF producing neurons to the same extent as controls (Ebbesson, unpublished observations). These central changes provide potential pathways for increased signalling through the LBP that increase the responsiveness to photic cues resulting in the elevation of circulating hormone levels essential for smoltification and seawater adaptation. Accordingly, juveniles reared on LL largely failed to show the smolt-related physiological changes (Fig. 1, Stefansson *et al.*, 2007). Gill mRNA levels of NKA isoforms α1a and α1b remain unchanged, as does NKCC. Consequently, gill NKA activity and NKAα and NKCC protein abundance are significantly lower in LL pseudo-smolts than in true smolts. The endocrine system also remains under-developed, with lower circulating levels of THs, GH and cortisol and lower expression of gill GH receptor. Taken together, deprivation of photoperiod information, e.g. continuous exposure to LL, disrupts smoltification by preventing the natural developmental program. Such photoperiod disruption has wide-ranging physiological consequences, suggesting that the photoperiod acts on key elements of the central regulatory system through its key role in the LBP.
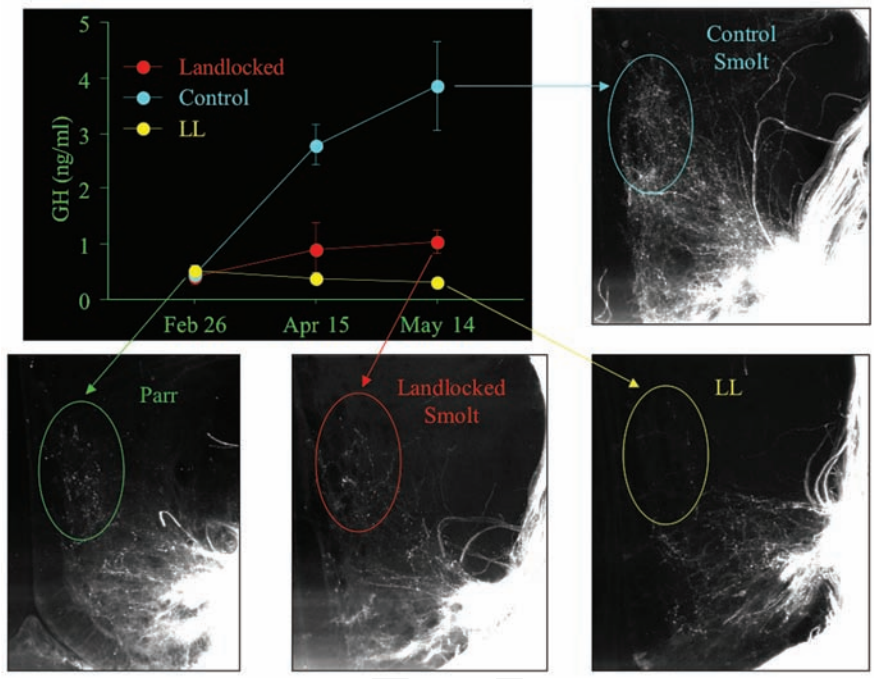
Fig. 5 Circulating growth hormone (GH) levels increase from parr in February to smolt in May in anadromous Atlantic salmon (*Salmo salar*), while they do not increase in either the continuous light (LL) or landlocked groups (A). The differences in hormone levels may be explained by degrees of brain development during this period. Retinal innervation into the preoptic nucleus (oval) in Atlantic salmon increases from parr in February (C) to control smolt in May (B). This increase is not observed in May in the landlocked salmon (D) or juveniles exposed to continuous light (LL; E; Ebbesson *et al.,* 2007; Stefansson *et al.,* 2007; Ebbesson *et al.,* unpublished data).

In Atlantic salmon culture, special attention has been given to the prospects of reducing the duration of the freshwater phase and transferring fish to sea out of season. Under standard farming conditions, smolts will be available only in spring (as either 1+ or 2+). However, it is possible to control and manipulate environmental conditions (temperature and specifically photoperiod) in order to produce off-season smolts, which may be transferred to seawater in the autumn of their first year. These smolts are referred to variously as 'half-year', 'underyearling' or 0+ smolts. The principle of off-season smolt production is to provide the juvenile salmon with a phase-advanced photoperiod signal of sufficient duration and amplitude to stimulate the completion of smoltification. Two strategies have been successful, one being the use of a segment of a simulated natural increase from approximately LD12:12 to LD24:0 with the natural rate of change (Berge *et al.*, 1995), the other strategy involves a 'square wave' photoperiod with a reduction in daylength from LD24:0 to LD12:12, keeping the fish

on LD12:12 'winter photoperiod' for several weeks, followed by a return to LD24:0, with monitoring of smolt development during the next few weeks (Arnesen *et al.*, 2003). The physiological changes associated with smoltification in 0+ smolts parallel those of ordinary 1+ smolts reared under commercial culture conditions (Sundell *et al.*, 2003), with improved seawater tolerance co-occurring with increased NKA activity (Berge *et al.*, 1995), elevated plasma GH (Björnsson *et al.*, 2000; Arnesen *et al.*, 2003), cortisol (Sundell *et al.*, 2003) and fully developed silvering and reduced condition factor.

Indeed, through judicious use of the two environmental control factors, photoperiod and temperature (see below), it is theoretically possible to produce successive cohorts of smolts throughout most of the year. In this scenario, specific temperature regimes can be used to either accelerate or delay growth while photoperiod can be used at the appropriate time to provide the necessary winter and spring signals to initiate and complete smoltification. The result will be a fully controlled, season-independent smolt production.

Temperature

Temperature is, together with daylength, the most important environmental factor controlling growth and developmental rates of juvenile salmonids. In natural environments, the annual temperature cycle defines the growth potential of the fish, and thereby the age at which juvenile salmonids reach the minimum size for initiation of smoltification. Accordingly, in European Atlantic salmon, smolt ages increases from 1+ in the southern part of their distribution range (Northern Spain) to 4+ or even 5+ in the rivers of Northern Norway and Russia. A similar picture is seen among Atlantic salmon populations in North America.

Temperature also influences the timing of smolt migration, directly and/or indirectly. In some rivers, smolt migration takes place as water temperature reaches a certain threshold temperature (e.g. River Ims, Jonsson & Ruud-Hansen, 1985). However, in contrast to the results suggesting a temperature threshold for initiation of downstream movement, recent findings by Zydlewski *et al.* (2005) suggest that d°C are a better indicator of the initiation and termination of downstream movement than is absolute temperature. Thermal cues are, however, probably not universal among salmon populations, as other river stocks appear to cue on to water flow and probably other environmental signals for the timing of their migration (see below). In rivers of Northern Sweden, with ice cover and temperatures close to zero until late spring (end of April), it has been suggested that the time window for smoltification and downstream migration is compressed, with full hypo-osmoregulatory ability still developing after the fish have entered the low-salinity Baltic sea (Schmitz *et al.*, 1994). The sea temperature that smolts experience at the time they reach salt water may be a strong selective force that has operated to affect the timing of emigration from seawater. Moving from south to north in Norway, smolt migration co-occurs with a sea temperature of approximately 9°C, which normally happens in April in the south and in June in the north

(NA Hvidsten, personal communication). A temperature of approximately 9°C is also the temperature suggested by Handeland *et al.* (2000) to cause the least osmotic disturbance in Atlantic salmon smolts during acclimation to full-strength seawater.

Under controlled conditions, through its influence on overall scope for growth, temperature controls the distribution of fish between the two growth modes (see discussion on bimodality above). Higher growth rate stimulates recruitment to the UM, and increases the number and size of potential smolts. Temperature also influences smoltification directly, by controlling the rate of physiological, morphological and behavioural development (Fig. 6, Björnsson *et al.,* 1989; McCormick *et al.*, 2000). Although temperature regulates the rate of development, it cannot act by itself as an independent zeitgeber, but rather appears to control the rate of response to photoperiodic cues (Solbakken *et al.*, 1994; McCormick *et al.,* 2002). Handeland *et al.* (2004) estimated a period of approximately 350 d°C between the onset of the smolt-related increase in gill NKA activity of Atlantic salmon and the peak in enzyme activity, providing a useful model for the prediction of completion of smoltification under various rearing temperatures. In a similar manner, temperature influences the duration of the period of peak smolt characters, e.g. high seawater tolerance, often referred to as the 'smolt window' (Fig. 6). The smolt window may ultimately be regulated by environmental influences on the neuroendocrine system (see above). Smolt characters are lost sooner when smolts are held in freshwater at higher temperatures, and this de-smoltification eventually prevents the smolts from acclimating to seawater. In the study of Handeland *et al.* (2004) the duration of the smolt window, defined as the period when gill NKA activity was > 90% of peak value, was estimated at 350 d°C. Similar models have been presented by Stefansson *et al.* (1998), Sigholt *et al.* (1998) and McCormick *et al.* (1999) who have proposed the duration of the smolt window in the range of 300-400 d°C, depending on the definition of the threshold level. Interestingly, recent findings by Zydlewski *et al.* (2005) on the behavioural aspects of smoltification seem to concur with physiological observations, with the opening and closing of the migratory window occurring at approximately the same number of d°C, independently of absolute temperature.

At the extremes, temperature may limit or even inhibit the completion of smoltification. Low temperatures may prevent the fish from responding to the increasing photoperiod by limiting the endocrine responsiveness of key endocrine tissues and target organs (McCormick *et al.*, 2000). Using the models outlined above, a temperature from April through June around 4°C would provide the juvenile salmon with approximately 350 d°C, and may be hypothesised as a lower temperature limit for smoltification. However, Handeland *et al.* (2004, see Fig. 6) and our own unpublished results suggest that different salmon strains are adapted to different temperature conditions, and smolt development differs among strains held at the same temperature, hence absolute limits may be difficult to define.

Studies on steelhead trout have suggested an upper limit for the completion of smoltification in the range of 13-15°C (Zaugg *et al.*, 1972; Zaugg & Wagner, 1973; Zaugg, 1981), based on the suppression of gill NKA activity above these temperatures.
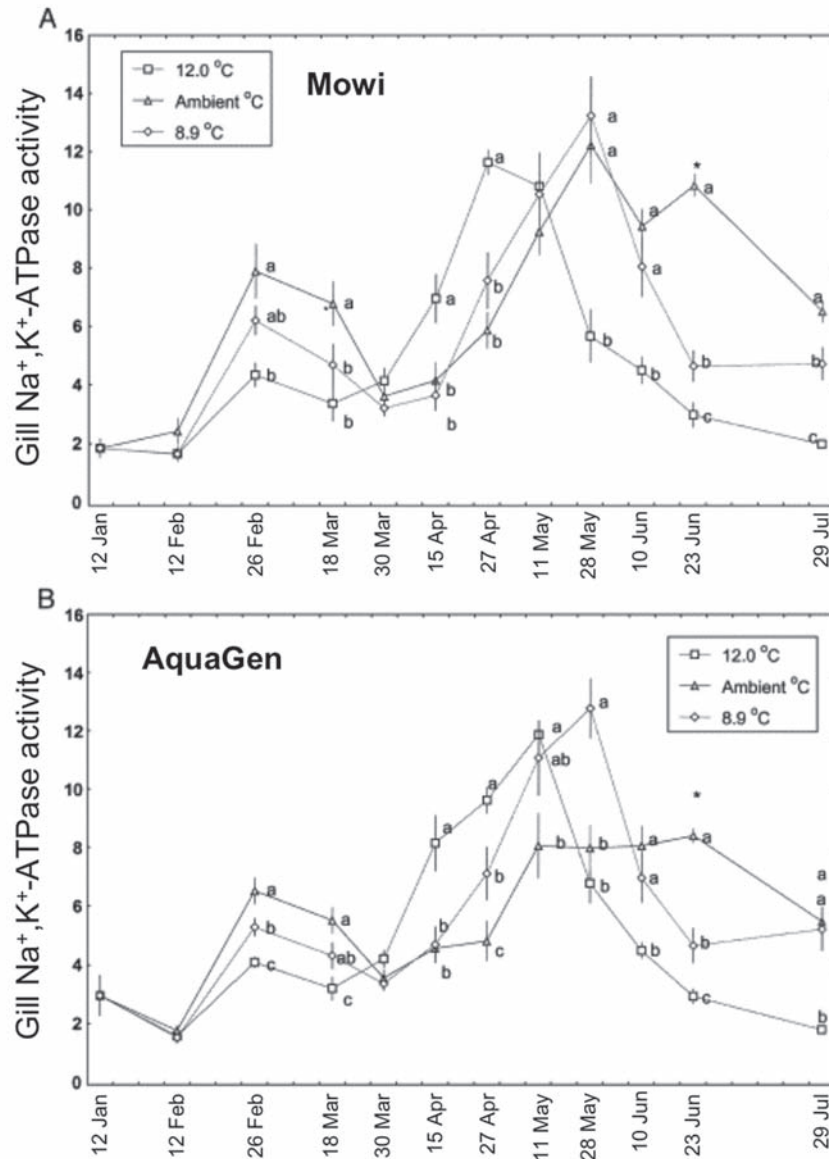
Fig. 6 Gill Na$^+$,K$^+$-ATPase activity in two farmed Norwegian strains of Atlantic salmon (*Salmo salar*) smolts (A: Mowi and B: AquaGen) at three temperatures (ambient, 8.9 and 12.0°C) during the period 12 January-29 July. Ambient temperature increased from 3°C in January to 10°C in June. Values are given as means (sem, n = 12). Significant differences among temperatures are indicated by different letters (p < 0.05). The asterisk indicates significant differences among strains (p < 0.05). From Handeland *et al.* (2004).

Other studies have failed to show such inhibition in other salmonid species. Atlantic salmon are able to adapt to seawater at temperatures as high as 19°C, although physiological stress and poor growth are observed at such high temperatures (Handeland *et al.*, 2000). Recent evidence has further shown that chinook salmon reared at temperatures above 17°C show impaired smoltification (Marine & Cech, 2004), suggesting that these temperatures are also restrictive for smoltification in this species. Taken together, studies to date indicate that temperature has a role in the timing of smolt development primarily through control of developmental rate, but that species-specific temperature limits may also exist.

Flow and Water Quality

The impact of flow on behaviour of Pacific and Atlantic salmon smolts has been reviewed previously (Hoar, 1988; McCormick *et al.,* 1998). Several studies have associated flow (water velocity) with smolt migration. Since smolts can move downstream both passively and actively, increased flow is likely to be detected as a factor in field studies of smolt migration simply through its effects on passive migration speed. The impact of flow on migration may relate to the initiation of migration and to the speed of migration. Fish may use other non-flow related signals such as water level, turbidity or novel chemical cues associated with flow events to initiate or increase their speed of migration.

Smolts are inherently more responsive than other life history stages of salmonids to a variety of external stressors, including poor water quality. Acid and associated aluminum toxicity have been shown to have severe impacts on smolts (Rosseland *et al.,* 2001). Direct mortality related to osmoregulatory failure in fresh water (loss of plasma ions) has been found at pH 5.0-5.5 with moderate to high aluminum levels (Staurnes *et al.*, 1996). At more moderate pH and aluminum conditions smolts survive in fresh water but incur a loss in gill NKA activity and salinity tolerance (Saunders *et al.,* 1983; Staurnes *et al.,* 1993). Reductions in salinity tolerance can occur within two days (Monette and McCormick, unpublished data) and are associated with loss of NKA activity and enzyme subunit disturbances (Kverneland, Nilsen and Stefansson, unpublished data). Although it remains to be demonstrated conclusively, aluminum exposure under acidic conditions may induce compensatory mechanisms for ion uptake that are in direct conflict with normal smoltification. Heavy metals also exert their toxic influence through impacts on ion regulation, and it stands to reason that smolts may be more sensitive than other life stages (or other species) due to the osmoregulatory change they undergo during smoltification, though to our knowledge this has not investigated.

As an endocrine-driven developmental event, smoltification is susceptible to endocrine disruption by contaminants that act through hormone signalling pathways. It has been known for some time that androgens are detrimental to development of normal smolt behaviour and physiology (Berglund *et al.,* 1994). In a retrospective study, Fairchild *et al.* (1999) found that areas of New Brunswick, Canada that had

been heavily sprayed for spruce budworm with a formulation that contained the xenoestrogen nonylphenol experienced reduced marine survival of smolts. Madsen *et al.* (1997) subsequently found that nonylphenol exposure can reverse the normal increases in gill CCs, NKA activity and salinity tolerance that occur during smoltification. These reductions in salinity tolerance are likely due to reductions in circulating levels of IGF-I (known to be important development mechanisms for salt secretion) that occurs after nonylphenol exposure (McCormick *et al.,* 2005). However, relatively high levels of aqueous nonylphenol are required to impact smolt development, levels that seem unlikely to occur in many river systems (Moore *et al.,* 2003). Lerner *et al.* (2006a) recently found that yolk-sac larvae are much more sensitive to nonylphenol than other life stages of Atlantic salmon, and that exposure to moderate levels (6 μg/L) can result in reduced salinity tolerance and salinity preference of smolts a full year after exposure. For a recent review of the influence of xenobiotics on salmonid eggs and larvae, see Finn (2007).

Other organic pollutants may also impact smolts, either through endocrine disruption or through more 'classic' toxic actions. Polychlorinated biphenyl (PCB) exposure reduces salinity tolerance, salinity preference and plasma TH (Lerner *et al.,* 2006b). Atrazine, a commonly used herbicide, has been found to reduce salinity tolerance of Atlantic salmon smolts (Waring & Moore 2004; Nieves-Puigdoller and McCormick, unpublished data). Contaminants may also interact to affect smolt development. Moore *et al.* (2003) found that atrazine at 1 μg/L or nonylphenol at 5 μg/L individually had no impact on smolts, but in combination the two compounds resulted in loss of salinity tolerance. A variety of contaminants interfere with olfaction in fishes, and compounds that affect olfaction may interfere both with the imprinting process that occurs primarily during smoltification, as well as the homing process during return of adults to their natal streams for spawning (Scholz *et al.,* 2000; Moore & Lower 2001; Jarrard *et al.,* 2004).

FUTURE PERSPECTIVES

Recent scientific progress concerning other anadromous species, e.g. American shad and striped bass (see Dadswell *et al.,* 1987, for a discussion of anadromous species), has raised the question of whether the smoltification is unique to salmonids, or whether similar processes can be found among other families of teleosts. The preparatory nature of the smoltification process sets it apart from the widespread capacity among euryhaline teleosts to adapt to gradual changes in salinity. Recent evidence obtained from American shad, however, suggests that these fish develop salinity tolerance while still in freshwater, with a loss of freshwater adaptations during migration (Zydlewski & McCormick, 2001), akin in many ways to the osmoregulatory changes occurring in smolts. Further studies comparing preparatory adaptations to seawater in various anadromous and euryhaline species are needed to determine whether the smoltification is indeed unique to salmonids and to provide a better understanding of the various physiological mechanisms used among teleosts in seawater adaptation.

Stefansson *et al.* (2003b) suggested specific research strategies that would be particularly fruitful for increasing our understanding of the smoltification process, including a better understanding of the variability in smoltification between and within strains, the importance of local adaptation for smolt quality and migration, challenges and actions under a global warming scenario, parameters defining and predicting smolt quality, and perspectives for improving hatchery smolt production. Further, functional genomics is another approach quickly making its way into smolt research. Large-scale gene expression/identification studies are likely to describe, on a very basic level of biological organisation, the control of smoltification. As more genes are identified in salmonids and other species and their products tied to specific functions (proteomics), an understanding of the biochemical and physiological processes involved in smoltification should emerge. Indeed, as we have pointed out, integrating information from other teleost models or target species will allow us to make rapid progress in traditional behavioural and physiological studies, as well as in functional genomic approaches to smoltification. Such work will hopefully also lead to development of useful tools such as microarrays for use in staging smolt development.

We would like to emphasise the importance of maintaining and strengthening the 'traditional' ecological, physiological, behavioural and environment-fish interaction studies at the organismal level. More work is required to understand the physiological and behavioural changes that occur in smolts in the wild and how these relate to survival and population dynamics. Such studies are critical for interpretation of findings at the molecular, cellular and organ levels, and are vital as input for the management of wild and reared salmon stocks.

References

Aas-Hansen O, Vijayan MM, Johnsen HK, Cameron C, Jorgensen EH (2005) Resmoltification in wild, anadromous Arctic char (*Salvelinus alpinus*): a survey of osmoregulatory, metabolic, and endocrine changes preceding annual seawater migration. Can J Fish Aquat Sci 62: 195-204

Allison WT, Veldhoen KM, Hawryshyn CW (2006) Proteomic analysis of opsins and thyroid hormone-induced retinal development using isotope-coded affinity tags (ICAT) and mass spectrometry. Mol Vision 12: 655-672

Arnesen AM, Toften H, Agustsson T, Stefansson SO, Handeland SO, Bjornsson BT (2003) Osmoregulation, feed intake, growth and growth hormone levels in 0+ Atlantic salmon (*Salmo salar* L.) transferred to seawater at different stages of smolt development. Aquaculture 222: 167-187

Baker BI, Bird DI, Buckingham JC (1996) In the trout, CRH and AVT synergize to stimulate ACTH release. Regulatory Peptides 67: 207-210

Barbour SE, Garside ET (1983) Some physiologic distinctions between freshwater and diadromous forms of the Atlantic salmon, *Salmo salar* L. Can J Zool 61: 1165-1170

Barron, MG (1986) Endocrine control of smoltification in anadromous salmonids. J Endocrinol 108: 313-319

Behnke RJ (1972 The systematics of salmonid fishes of recently glaciated lakes. J Fish Res Board Can 29: 639-671

Bell JG, Sargent JR (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218: 491-499

Bell JG, Tocher DR, Farndale BM, Cox DI, McKinney RW, Sargent JR (1997) The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr–smolt transformation. Lipids 32: 515-525

Benfey TJ, Saunders RL, Knox DE and Harmon PR (1994) Muscle ornithine decarboxylase activity as an indication of recent growth in pre-smolt Atlantic salmon, *Salmo salar*. Aquaculture 121: 125-135

Berg M (1953) A relict salmon, *Salmo salar* L., called "småblank" from the river Namsen, North Tröndelag. Acta Boreal Sci 6: 1-19

Berg OK (1985) The formation of non-anadromous populations of Atlantic salmon, *Salmo salar* L., in Europe. J Fish Biol 27: 805-815

Berge ÅI, Berg A, Fyhn HJ, Barnung T, Hansen T, Stefansson SO (1995) Development of salinity tolerance in underyearling smolts of Atlantic salmon (*Salmo salar*) reared under different photoperiods. Can J Fish Aquat Sci 52: 243-251

Berglund I, Lundqvist H, Fängstam H (1994) Downstream migration of immature salmon (*Salmo salar*) smolts blocked by implantation of the androgen 11-ketoandrostenedione. Aquaculture 121: 269-276

Bern HA (1978) Endocrinological studies on normal and abnormal salmon smoltification. In: Gaillard PJ, Boer HH (eds) Comparative Endocrinology. Elsevier/North Holland Biomedical Press, Amsterdam, p 77-100

Birt TP, Green JM (1986) Parr–smolt transformation in female and sexually mature male anadromous and nonanadromous Atlantic salmon, *Salmo salar*. Can J Fish Aquat Sci 43: 680-686

Birt TP, Green JM (1993) Acclimation to seawater of dwarf nonanadromous Atlantic salmon, *Salmo salar*. Can J Zool 71: 1912-1916

Birt TP, Green JM, Davidson WS (1991) Contrasts in development and smolting of genetically distinct sympatric anadromous and nonanadromous Atlantic salmon, *Salmo salar*. Can J Zool 69: 2075-2084

Björnsson BT (1997) The biology of salmon growth hormone: from daylight to dominance. Fish Physiol Biochem 17: 9-24

Björnsson BT, Thorarensen H, Hirano T, Ogasawara T, Kristinsson JB (1989) Photoperiod and temperature affect plasma growth hormone levels, growth, condition factor and hypoosmoregulatory ability of juvenile Atlantic salmon (*Salmo salar*) during parr-smolt transformation. Aquaculture 82: 77-91

Björnsson BT, Taranger GL, Hansen T, Stefansson SO, Haux C (1994) The interrelation between photoperiod, growth hormone, and sexual maturation of adult Atlantic salmon (*Salmo salar*). Gen Comp Endocrinol 93: 70-81

Björnsson BT, Stefansson SO, Hansen T (1995) Photoperiod regulation of plasma growth hormone levels during parr-smolt transformation of Atlantic salmon: implications for hypoosmoregulatory ability and growth. Gen Comp Endocrinol 100: 73-82

Björnsson BT, Stefansson GV, Berge AI, Hansen T, Stefansson SO (1998) Circulating growth hormone levels in Atlantic salmon smolts following seawater transfer: effects of photoperiod regime, salinity, duration of exposure and season. Aquaculture 168: 121-137

Björnsson BT, Hemre GI, Bjornevik M, Hansen T (2000) Photoperiod regulation of plasma growth hormone levels during induced smoltification of underyearling Atlantic salmon. Gen Comp Endocrinol 119: 17-25

Björnsson BT, Johansson V, Benedet S, Einarsdottir IE, Hildahl J, Agustsson T, Jonsson E (2002) Growth hormone endocrinology of salmonids: regulatory mechanisms and mode of action. Fish Physiol Biochem 27: 227-242

Blake RL, Roberts FL, Saunders RL (1984) Parr–smolt transformation of Atlantic salmon (*Salmo salar*): activities of two respiratory enzymes and concentrations of mitochondria in the liver. Can J Fish Aquat Sci 41: 199-203

Browman HI, Hawryshyn CW (1994) The developmental trajectory of ultraviolet photosensitivity in rainbow trout is altered by thyroxine. Vision Res 34: 1397-1406

Burton MP, Idler DR (1984) Can Newfoundland landlocked salmon, *Salmo salar* L., adapt to sea water? J Fish Biol 24: 59–64

Canosa LF, Chang JP, Peter RE (2007) Neuroendocrine control of growth hormone in fish. Gen Comp Endocrinol 151: 1-26

Chernitsky AG (1986) Quantitative evaluation of the degree of parr-smolt transformation in wild smolts and hatchery juveniles of Atlantic salmon (*Salmo salar* L.) by SDH activity of chloride cells. Aquaculture 59: 287-297

Chernitsky AG, Loenko, AA (1983) The osmoregulatory system and possible ways of differentiation in ecological forms of Atlantic salmon, *Salmo salar* (Salmonidae). J Ichthyol 23: 84–94

Collie NL, Bern HA (1982) Changes in intestinal fluid transport associated with smoltification and seawater adaptation in Coho salmon *Oncorhynchus kisutch* (Walbaum). J Fish Biol 21: 337-348

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Shrimpton JM, Van der Kraak G, Farrell AP (2006) Physiology of individual late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. Can J Fish Aquat Sci 63: 1469-1480

Cowley DJ, Sheridan MA, Hoffnagle TL, Fivizzani AJ, Barton BA, Eilertson CD (1994) Changes in lipid metabolism and plasma concentrations of thyroxine, cortisol, and somatostatin in land-locked chinook salmon, *Oncorhynchus tshawytscha*, during smoltification. Aquaculture 121: 147-155

Dadswell MJ, Melvin GD, Williams PJ, Themelis DE (1987) Influences of origin, life history, and chance on the Atlantic coast migration of American shad. Am Fish Soc Symp 1: 313-330

Dahl K (1928) The Blege or dwarf-salmon. Skr Utg Det Norske Videnskabsakad 1927, Oslo (in Norwegian)

Durif C, Dufour S, Elie P (2005) The silvering process of *Anguilla anguilla*: a new classification from the yellow resident to the silver migrating stage J Fish Biol 66: 1025-1043

Duston J, Saunders RL, Knox DE (1991) Effects of increases in fresh-water temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 48: 164-169

Eales JG (1995) Regulation and measurement of thyroidal status in fish. Netherlands J Zool 45:175-180

Eales JG, Morin PP, Tsang P, Hara TJ (1993) Thyroid hormone deiodination in brain, liver, gill, heart, and muscle of Atlantic salmon (*Salmo salar*) during photoperiodically-induced parr–smolt transformation, II. Outer and inner-ring 3,5,3'-triiodo-L-thyronine and 3,3',5'-triiodo-L-thyronine reverse T3 deiodination. Gen Comp Endocrinol 90: 157-167

Ebbesson LOE (2000) Temporal dynamics of brain and hormone changes during parr-smolt transformation in salmon. PhD thesis, Lund University, Sweden

Ebbesson LOE, Holmqvist B, Östholm T, Ekström P (1992) Transient serotonin-immunoreactive neurons coincide with a critical period of neural development in coho salmon (*Oncorhynchus kisutch*). Cell Tissue Res 268: 389-392

Ebbesson LOE, Drew K, Ebbesson SOE (1994) Brain development during smolt transformation in salmon may be affected by environmental conditions. Soc Neurosci Abstracts 20: 1301

Ebbesson LOE, Deviche P, Ebbesson SOE (1996a) Distribution and changes in mu- and kappa-opiate receptors during the midlife neurodevelopmental period of coho salmon, *Oncorhynchus kisutch*. J Comp Neurol 366: 448-464

Ebbesson LOE, Ekström P, Ebbesson SOE, Stefansson SO, Holmqvist B (2003) Neural circuits and their structural and chemical reorganization in the light-brain-pituitary axis during parr-smolt transformation in salmon. Aquaculture 222: 59-70

Ebbesson LOE, Ebbesson SOE, Nilsen TO, Stefansson SO, Holmqvist B (2007) Exposure to continuous light disrupts retinal innervation of the preoptic nucleus during parr-smolt transformation in Atlantic salmon. Aquaculture (in press)

Ebbesson SOE (1980) The parcellation theory and its relation to interspecific variability in brain organization evolutionary and ontogenetic development and neuronal plasticity. Cell Tissue Res 213: 179-212

Ebbesson SOE, Smith J, Co C, Ebbesson LOE (1996b) Transient alterations in neurotransmitter levels during a critical period of neural development in coho salmon (*Oncorhynchus kisutch*). Brain Res 742: 339-342

Eriksson LO, Lundqvist H (1982) Circannual rhythms and photoperiod regulation of growth and smolting in Baltic salmon (*Salmo salar* L.). Aquaculture 28: 113-121.

Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev 85: 97-177

Fairchild WL, Swansburg EO, Arsenault JT, Brown SB (1999) Does an association between pesticide use and subsequent declines in catch of Atlantic salmon (*Salmo salar*) represent a case of endocrine disruption? Env Health Perspectives 107: 349-357

Finn RN (2007) The physiology and toxicology of salmonid embryos and larvae in relation to water quality criteria. Aquat Toxicol 81: 337-354

Fontaine M, Marchelidon J (1971) Amino acid contents of the brain and the muscle of young salmon (*Salmo salar* L.) at parr and smolt stages. Comp Biochem Physiol 40: 127-134

Foster AR, Houlihan DF, Gray C, Medale F, Fauconneau B, Kaushikj SJ, Le Bail PY (1991) The effects of ovine growth hormone on protein turnover in rainbow trout. Gen Comp Endocrinol 82: 111-120

Fyhn UEH, Clarke WC, Whitler RE (1991) Hemoglobins in smoltifying Chinook salmon, *Oncorhynchus tshawytscha*, subjected to photoperiod control. Aquaculture 95: 359-372

Garber MJ, DeYonge KG, Byatt JC, Lellis WA, Honeyfield DC, Bull RC, Schelling GT, Roeder RA (1995) Dose-response effects of recombinant bovine somatotropin (Posilac) on growth performance and body composition of two-year-old rainbow trout (*Oncorhynchus mykiss*). J Anim Sci 73: 3216–3222

Gill JA, Sumpter JP, Donaldson EM, Dye HM, Souza L, Berg T, Wypych J, Langley K (1985) Recombinant chicken and bovine growth-hormone accelerate growth in aquacultured juvenile Pacific salmon, *Oncorhynchus kisutch*. Biotechnology 3: 643-646

Gross M (1987) Evolution of diadromy in fishes. Am Fish Soc Symp 1: 14-25

Handeland SO, Stefansson SO (2001) Photoperiod control and influence of body size on off-season parr-smolt transformation and post-smolt growth. Aquaculture 192: 291-307

Handeland SO, Stefansson SO (2002) Effects of salinity acclimation on pre-smolt growth, smolting and post-smolt performance in off-season Atlantic salmon smolts (*Salmo salar* L.). Aquaculture 209: 125-137

Handeland SO, Järvi T, Fernö A, Stefansson SO (1996) Osmotic stress, antipredator behaviour, and mortality of Atlantic salmon (*Salmo salar*) smolts. Can J Fish Aquat Sci 53: 2673-2680

Handeland SO, Berge A, Björnsson BT, Lie Ø, Stefansson SO (2000) Seawater adaptation by out-of-season Atlantic salmon (*Salmo salar* L.) smolts at different temperatures. Aquaculture 181: 377-396

Handeland SO, Björnsson BT, Arnesen AM, Stefansson SO (2003) Seawater adaptation and growth of post-smolt Atlantic salmon (*Salmo salar*) of wild and farmed strains. Aquaculture 233: 367-384

Handeland SO, Wilkinson E, Sveinsbø B, McCormick SD, Stefansson SO (2004) Temperature influence on the development and loss of seawater tolerance in two fast-growing strains of Atlantic salmon. Aquaculture 233: 513-529

Heggenes J, Metcalfe NB (1991) Bimodal size distributions in wild juvenile Atlantic salmon populations and their relationship with age at smolt migration. J Fish Biol 39: 905-907

Herbert NA, Armstrong JD, Björnsson BT (2001) Evidence that growth hormone-induced elevation in routine metabolism of juvenile Atlantic salmon is a result of increased spontaneous activity. J Fish Biol 59: 754-757

Higgs DA, Donaldson EM, Dye HM, McBride JR (1975) Preliminary investigation of effect of bovine growth-hormone on growth and muscle composition of coho salmon (*Oncorhynchus kisutch*). Gen Comp Endocrinol 27: 240-253

Hiroi J, Kaneko T, Tanaka M (1999) *In vivo* sequential changes in chloride cell morphology in the yolk-sac membrane of Mozambique tilapia (*Oreochromis mossambicus*) embyros and larvae during seawater adaptation. J. Exp Biol 202: 3485-3495

Hoar WS (1976) Smolt transformation: evolution, behaviour, and physiology. J Fish Res Board Can 33: 1234-1252

Hoar WS (1988) The physiology of smolting salmonids. In: Hoar WS, Randall DJ (eds) Fish Physiology, Vol 11B, Academic Press, London, p 275-343

Hofmann MH, Meyer DL (1993) Visual-evoked responses in the salmon telencephalon change during smolt transformation. Neurosci Lett 155: 234-236

Holmqvist BI, Östholm T, Ekström P (1992) Retinohypothalamic projections and suprachiasmatic nucleus of the teleost brain. In: Ali MA (ed) Rhythms in Fish. Plenum Press, New York, p 293-318

Holmqvist BI, Östholm T, Ekström P (1994) Neuroanatomical analysis of the visual and hypophysiotrophic systems in Atlantic salmon (*Salmo salar*) with emphasis on possible mediators of photoperiodic cues during parr-smolt transformation. Aquaculture 121: 1-12

Hutchings JA, Jones MEB (1998) Life history variation and growth rate thresholds for maturity in Atlantic salmon, *Salmo salar*. Can J Fish Aquat Sci 55: 22-47

Hutchison MJ, Iwata M (1998) Effect of thyroxine on the decrease of aggressive behaviour of four salmonids during the parr-smolt transformation. Aquaculture 168:169-175

Jakobsson S, Mayer I, Borg, B (1997) Androgen binding in the gills of Atlantic salmon, *Salmo salar*, mature male parr, and immature male smolts. J Exp Biol 278: 391-394

Jarrard HE, Delaney KR, Kennedy CJ (2004) Impacts of carbamate pesticides on olfactory neurophysiology and cholinesterase activity in coho salmon (*Oncorhynchus kisutch*). Aquat Toxicol 69: 133-148

Ji H, Bradley TM, Tremblay, GC (1996) Lactate-dependent gluconeogenesis and atractyloside-sensitive flux through pyruvate carboxylase are reduced during smoltification of Atlantic salmon (*Salmo salar*). J Exp Zool 276: 375–386

Johansson V, Winberg S, Hall D, Jönsson E, Björnsson BT (2004) Peripherally administered growth hormone affects behaviour and brain dopaminergic activity in rainbow trout. Horm Behav 46: 436-443

Johansson V, Winberg S, Björnsson BT (2005) Growth hormone-induced stimulation of swimming and feeding behaviour of rainbow trout is abolished by the D1 dopamine antagonist SCH23390. Gen Comp Endocrinol 141: 58-65

Johnsson JI, Björnsson BT (1994) Growth hormone increases growth rate, appetite and dominance in juvenile rainbow trout, *Oncorhynchus mykiss*. Animal Behav 48: 177-186

Johnsson JI, Jönsson E, Björnsson BT (1996a) Dominance, nutritional status and growth hormone levels in rainbow trout (*Oncorhynchus mykiss*). Horm Behav 30: 13-21

Johnsson JI, Petersson E, Jönsson E, Björnsson BT, Järvi T (1996b) Domestication and growth hormone alter growth patterns and anti-predator behaviour in juvenile brown trout, *Salmo trutta*. Can J Fish Aquat Sci 53: 1546-1554

Johnsson JI, Petersson E, Jönsson E, Järvi T, Björnsson BT (1999) Growth hormone induced effects on mortality, energy status and growth: a field study on brown trout (*Salmo trutta*). Funct Ecol 13: 514-522

Johnsson JI, Jönsson E, Petersson E, Järvi T, Björnsson BT (2000) Fitness-related effects of growth investment in brown trout under field and hatchery conditions. J Fish Biol 57: 326-336

Johnston CE, Eales JG (1967) Purines in the integument of Atlantic salmon (*Salmo salar*) during parr-smolt transformation. J Fish Res Board Can 24: 955-964

Jonsson B, Ruud-Hansen J (1985) Water temperature as the primary influence on timing of seaward migrations of Atlantic salmon (*Salmo salar*) smolts. Can J Fish Aquat Sci 42: 593-595

Jönsson E, Johnsson JI, Björnsson BT (1996) Growth hormone increases predation exposure of rainbow trout. Proc Royal Soc London B 263: 647-651

Jönsson E, Johnsson JI, Björnsson BT (1998) Growth hormone increases aggressive behaviour in juvenile rainbow trout. Horm Behav 33: 9-15

Jönsson E, Johansson V, Björnsson BT, Winberg S (2003) Central nervous system actions of growth hormone on brain monoamine levels and behaviour of juvenile rainbow trout. Horm Behav 43: 367-374

Kristinsson JB, Saunders RL, Wiggs AJ (1985) Growth dynamics during the development of bimodal length-frequency distribution in juvenile Atlantic salmon (*Salmo salar* L.). Aquaculture 45: 1-20

Kudo H, Tsuneyoshi Y, Nagae M, Adachi S, Yamauchi K, Ueda U, Kawamura H (1994) Detection of thyroid hormone receptors in the olfactory system and brain of wild masu salmon, *Oncorhynchus masou* (Brevoort), during smolting by in vitro autoradiography. Aquaculture and Fisheries Management 25:171-182

Kurokawa T (1990) Influence of the date and body size at smoltification and subsequent growth rate and photoperiod on desmoltification in underyearling masu salmon (*Oncorhynchus masou*). Aquaculture 86: 209-218

Langdon JS, Thorpe JE, Roberts RJ (1984) Effects of cortisol and ACTH on gill Na+/K+-ATPase, SDH and chloride cells in juvenile Atlantic salmon *Salmo salar* L. Comp Biochem Physiol 77: 9-12

Langdon JS, Thorpe JE (1985) The ontogeny of smoltification: Developmental patterns of gill Na+/K+-ATPase, SDH, and chloride cells in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture 45: 83-95

Lema SC, Nevitt GA (2004) Evidence that thyroid hormone induces olfactory cellular proliferation in salmon during a sensitive period for imprinting. J Exp Biol 207: 3317-3327

Leonard JBK, McCormick SD (2001) Metabolic enzyme activity during smolting in stream- and hatchery-reared Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 58: 1585-1593

Lerner DT, Björnsson BT, McCormick SD (2006a) Early exposure to environmental estrogens disrupts development of seawater adaptation in salmon. Env Health Perspectives (in press)

Lerner DT, Björnsson BT, McCormick, SD (2006b) Effects of aqueous exposure to polychlorinated biphenyls (Arocolor 1254) on physiology and behaviour of smolt development of Atlantic salmon. Aquat Toxicol 81: 329-336

Lewis KA, Swanson P, Sower SA (1992) Changes in brain gonadotropin-releasing hormone pituitary and plasma gonadotropins and plasma thyroxine during smoltification in chinook salmon (*Oncorhynchus tshawytscha*). Gen Comp Endocrinol 87: 461-470

Li, HO, Yamada, J (1992) Changes of the fatty acid composition in smolts of masu salmon (*Oncorhynchus masou*), associated with desmoltification and sea-water transfer. Comp Biochem Physiol 103: 221-226

Lignot JH, Cutler CP, Hazon N, Cramb G (2002) Immunolocalisation of aquaporin 3 in the gill and the gastrointestinal tract of the European eel (*Anguilla anguilla* L.). J Exp Biol 205: 2653–2663

Lubin RT, Rourke AW, Bradley TM (1989) Ultrastructural alteration in branchial chloride cells of Atlantic salmon, *Salmo salar*, during the parr-smolt transformation and early development in sea water. J Fish Biol 34: 259-272

Lundqvist H, Borg B, Berglund, I (1989) Androgens impair seawater adaptability in smolting Baltic salmon (*Salmo salar*). Can J Zool 67: 1733-1736

Mackie P, Wright PA, Glebe BD, Ballantyne JS (2005) Osmoregulation and gene expression of Na+/K+ ATPase in families of Atlantic salmon (*Salmo salar*) smolts. Can J Fish Aquat Sci 62: 2661-2672

Madsen SS, Mathiesen AB, Korsgaard B (1997) Effects of 17 beta-estradiol and 4-nonylphenol on smoltification and vitellogenesis in Atlantic salmon (*Salmo salar*). Fish Physiol Biochem 17: 303-312

Madsen SS, Skovbolling S, Nielsen C, Korsgaard B (2004) 17-beta estradiol and 4-nonylphenol delay smolt development and downstream migration in Atlantic salmon, *Salmo salar*. Aquat Toxicol 68: 109-120

Marine KR, Cech, JJ (2004) Effects of high water temperature on growth, smoltification, and predator avoidance in Juvenile Sacramento River Chinook salmon. North Am J Fish Management 24: 198-210

Markert JR, Higgs DA, Dye HM, MacQuarrie DW (1977) Influence of bovine growth-hormone on growth-rate, appetite, and food conversion of yearling coho salmon (*Oncorhynchus kisutch*) fed two diets of different composition. Can J Zool 55: 74-83

Marshall WS (2002) Na^+, Cl^-, Ca^{2+} and Zn^{2+} transport by fish gills: Retrospective review and prospective synthesis. J Exp Zool 293: 264-283

Martin-Smith KM, Armstrong JD, Johnsson JI, Björnsson BT (2004) Growth hormone increases growth and dominance of wild juvenile Atlantic salmon without affecting space use. J Fish Biol 65: 156-172

Maule AG, Tripp RA, Kaattari SL, Schreck CB (1989) Stress alters immune function and disease resistance in Chinook salmon (*Oncorhynchus tshawytscha*). J Endocrinol 120: 135-142

Maxime V, Boeuf G, Pennec JP, Peyraud C (1989) Comparative study of the energetic metabolism of Atlantic salmon (*Salmo salar*) parr and smolts. Aquaculture 82: 163-171

Mazurais D, Le Drean G, Brierley I, Anglade I, Bromage N, Williams LM, Kah O (2000) Expression of clock gene in the brain of rainbow trout: Comparison with the distribution of melatonin receptors. J Comp Neurol 422: 612-620

McCormick SD (1994) Ontogeny and evolution of salinity tolerance in anadromous salmonids: Hormones and heterochrony. Estuaries 17: 26-33

McCormick SD (2001) Endocrine control of osmoregulation in teleost fish. Am Zool 41: 781-794

McCormick SD, Saunders RL (1987) Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. Am Fish Soc Symp 1: 211-299

McCormick SD, Saunders RL, Henderson EB, Harmon PR (1987) Photoperiod control of parr-smolt transformation in Atlantic salmon (*Salmo salar*): changes in salinity tolerance, gill Na^+,K^+-ATPase activity and plasma thryoid hormones. Can J Fish Aquat Sci 45: 1462-1468

McCormick SD, Björnsson BT, Sheridan M, Eilertson C, Carey JB, O'Dea M (1995) Increased daylength stimulates plasma growth hormone and gill Na^+,K^+-ATPase in Atlantic salmon (*Salmo salar*). J Comp Physiol 165: 245-254

McCormick SD, Hansen LP, Quinn TP, Saunders RL (1998) Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 55: 77-92

McCormick SD, Cunjak RA, Dempson B, O'Dea MF, Carey JB (1999) Temperature-related loss of smolt characteristics in Atlantic salmon (*Salmo salar*) in the wild. Can J Fish Aquat Sci 56: 1649-1658

McCormick SD, Moriyama S, Björnsson BT (2000) Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. Am J Physiol 278: R1352-R1361

McCormick SD, Björnsson BT, Moeckel AM, Lerner DT, O'Dea MF (2001) Endocrine pathways for the disruption of the parr-smolt transformation by estradiol and nonylphenol. Am Zool 41: 1518-1518

McCormick SD, Shrimpton JM, Moriyama S, Björnsson BT (2002) Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon. J Exp Biol 205: 3553-3560

McCormick SD, O'Dea MF, Moeckel AM, Lerner DT, Björnsson BT (2005) Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic salmon by 4-nonylphenol and 17 beta-estradiol. Gen Comp Endocrinol 142: 280-288

McCormick SD, Shrimpton JM, Moriyama S, Björnsson BT (2007) Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: a possible developmental basis for smolting. Aquaculture (in press)

McDowall RM (1987) The occurrence and distribution of diadromy among fishes. Am Fish Soc Symp 1: 1-13

McKeown BA, Leatherland JF, John TM (1975) The effect of growth hormone and prolactin on the mobilization of free fatty acids and glucose in the kokanee salmon, *Oncorhynchus nerka*. Comp Biochem Physiol 50: 425-430

McLean E, Donaldson EM (1993) The role of growth hormone in growth of poikilotherms. In: Schreibman MP, Scanes CG, Pang PKT (eds) The Endocrinology of Growth, Development and Metabolism in Vertebrates. Academic Press, NY, p 43-71

Melingen GO, Wergeland HI (2000) Serum protein and IgM profiles in connection with the smolting and vaccination of out-of-season Atlantic salmon (*Salmo salar* L.). Aquaculture 188: 189-201

Melingen GO, Pettersen EF, Wergeland HI (2002) Leucocyte populations and responses to immunization and photoperiod manipulation in Atlantic salmon (*Salmo salar* L.) 0+ smolt. Aquaculture 214: 381-396

Metcalfe NB, Thorpe JE (1992) Anorexia and defended energy levels in over-wintering juvenile salmon. J Animal Ecol 61: 175-181

Metcalfe NB, Huntingford FA, Thorpe, JE (1986) Seasonal changes in feeding motivation of juvenile Atlantic salmon *Salmo salar*. Can J Zool 64: 2439-2446

Metcalfe NB, Huntingford FA, Thorpe JE (1992) Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon *Salmo salar*. J Animal Ecol 57: 463-474

Moore A, Lower N (2001) The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Comp Biochem Physiol 129: 269-276

Moore A, Scott AP, Lower N, Katsiadaki I, Greenwood L (2003) The effects of 4-nonylphenol and atrazine on Atlantic salmon (*Salmo salar* L.) smolts. Aquaculture 222: 253-263

Morin PP, Hara TJ, Eales JG (1993) Thyroid hormone deiodination in brain, liver, gill, heart, and muscle of Atlantic salmon (*Salmo salar*) during photoperiodically-induced parr-smolt transformation I. Outer and inner-ring thyroxine deiodination. Gen Comp Endocrinol 90: 142-156

Morin PP, Andersen Ø, Haug E, Døving KB (1994) Changes in serum free thyroxine, prolactin, and olfactory activity during induced smoltification in Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 51:1985-1992

Morin PP, Hara TJ, Eales JG (1995) T-4 depresses olfactory responses to L-alanine and plasma T-3 and T-3 production in smoltifying Atlantic salmon. Am J Physiol 38: R1434-R1440

Morin PP, Winberg S, Nilsson GE, Hara TJ, Eales JG (1997) Effects of L-thyroxine on brain monoamines during parr-smolt transformation of Atlantic salmon (*Salmo salar* L). Neurosci Lett 224: 216-218

Moriyama S (1995) Increased plasma insulin-like growth-factor-I (IGF-I) following oral and intraperitoneal administration of growth hormone to rainbow trout, *Oncorhynchus mykiss*. Growth Regulation 5: 164-167

Moriyama S, Swanson P, Nishii M, Takahashi A, Kawauchi H, Dickhoff WW, Plisetskaya EM (1994) Development of a homologous radioimmunoassay for coho salmon insulin-like growth-factor-I. Gen Comp Endocrinol 96: 149-161

Mustafa T, Srivastava KC (1989) Prostaglandins (eicosanoids) and their role in ectothermic organisms. Adv Comp Environ Physiol 5: 157-207

Neregård L, Sundt-Hansen L, Hindar K, Einum S, Johnsson JI, Devlin RH, Fleming I, Björnsson BT (2007) Selective breeding of Atlantic salmon affects growth hormone action on growth and lipid metabolism. Can J Aquat Fish Sci (in press)

Nicieza AG, Reyesgavilan FG, Brana F (1994) Differentiation in juvenile growth and bimodality patterns between northern and southern populations of Atlantic salmon (*Salmo salar* L.). Can J Zool 72: 1603-1610

Nielsen C, Madsen SS, Björnsson BT (1999) Changes in branchial and intestinal osmoregulatory mechanisms and growth hormone levels during smolting in hatchery-reared and wild brown trout. J Fish Biol 54: 799-818

Nilsen TO, Ebbesson LOE, Stefansson SO (2003) Smolting in anadromous and landlocked strains of Atlantic salmon (*Salmo salar*). Aquaculture 222: 71-82

Nilsen TO, Ebbesson LOE, Madsen SS, McCormick SD, Andersson E, Björnsson BT, Prunet P, Stefansson SO (2007) Differential expression of gill Na^+, K^+-ATPase α and β subunits, Na^+,K^+,$2Cl^-$ cotransporter and CFTR anion channel in Juvenile anadromous and landlocked Atlantic salmon (*Salmo salar*). J Exp Biol (in press)

Nordgarden U, Björnsson BT, Hansen T (2007) Developmental stage of Atlantic salmon parr regulates pituitary GH secretion and parr–smolt transformation Aquaculture 264: 441–448

Parhar IS, Iwata M (1996) Intracerebral expression of gonadotropin-releasing hormone and growth hormone-releasing hormone is delayed until smoltification in the salmon. Neurosci Res 26: 299-308

Payan P, Girard JP, Mayergostan N (1984) Branchial ion movements in teleosts – the roles of respiratory and chloride cells. In: Hoar WS, Randall DJ (eds) Fish Physiology, Vol 10B, Academic Press, London, p 39-63

Peake S, McKinley RS (1998) A re-evaluation of swimming performance in juvenile salmonids relative to downstream migration. Can J Fish Aquat Sci 55: 682-687

Pelis RM, Zydlewski J, McCormick SD (2001) Gill Na+-K+-2Cl(-) cotransporter abundance and location in Atlantic salmon: effects of seawater and smolting. Am J Physiol 280: R1844-R1852

Pisam M, Prunet P, Boeuf G, Rambourg A (1988) Ultrastructural features of chloride cells in the gill epithelium of the Atlantic salmon, *Salmo salar*, and their modifications during smoltification. Am J Anat 183: 235-244

Plisetskaya EM, Moon TW, Larsen DA, Foster GD, Dickhoff WW (1994) Liver glycogen, enzyme activities, and pancreatic hormones in juvenile Atlantic salmon (*Salmo salar*) during their first summer in seawater. Can J Fish Aquat Sci 51: 567–576

Poupa O, Gesser H, Jonsson S, Sullivan L (1974) Coronary-supplied compact shell of ventricular myocardium in salmonids: Growth and enzyme pattern. Comp Biochem Physiol 48: 85-88

Power DM, Llewellyn L, Faustino M, Nowell MA, Björnsson BT, Einarsdottir IE, Canario AVM, Sweeney GE (2001) Thyroid hormones in growth and development of fish. Comp Biochem Physiol 130: 447-459

Power G (1958) The evolution of the freshwater races of the Atlantic salmon (*Salmo salar* L.). Arctic 11: 86– 92

Richards JG, Semple JW, Bystriansky JS, Schulte PM (2003) Na^{+}/K^{+}-ATPase α-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. J Exp Biol 206: 4475-4486

Richardson SJ, Monk JA, Shepherdley CA, Ebbesson LOE, Sin F, Power DM, Frappell PB, Kohrle J, Renfree MB (2005) Developmentally regulated thyroid hormone distributor proteins in marsupials, a reptile, and fish. Am J Physiol 288: R1264-R1272

Rosseland BO, Kroglund F, Staurnes M, Hindar K, Kvellestad A (2001) Tolerance to acid water among strains and life stages of Atlantic salmon (*Salmo salar* L.). Water, Air and Soil Pollution 130: 899-904

Sargent JR, Bell JG, McEvoy L, Tocher DR, Estevez A (1999) Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177: 191-199

Saunders RL, Henderson EB (1970) Influence of photoperiod on smolt development and growth of Atlantic salmon (*Salmo salar*). J Fish Res Board Can 27: 1295-1311

Saunders RL, Henderson EB (1978) Changes in gill ATPase activity and smolt status of Atlantic salmon (*Salmo salar*). J Fish Res Board Can 35: 1542-1546

Saunders RL, Henderson EB, Harmon PR, Johnston CE and Eales JG (1983) Effects of low environmental pH on smolting of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 40: 1203-1211

Schmitz M (1995) Seasonal changes in hypoosmoregulatory ability in landlocked and anadromous populations of Arctic charr, *Salvelinus alpinus*, and Atlantic salmon, *Salmo salar*. Environ Biol Fish 42: 401–412

Schmitz M, Berglund I, Lundqvist H, Björnsson BT (1994) Growth hormone response to seawater challenge in Atlantic salmon, *Salmo salar*, during parr-smolt transformation. Aquaculture 121: 209-221.

Scholz NL, Truelove NK, French BL, Berejikian BA, Quinn TP, Casillas E, Collier TK (2000) Diazinon disrupts antipredator and homing behaviours in chinook salmon (*Oncorhynchus tshawytscha*). Can J Fish Aquat Sci 57:1911-1918

Seddiki H, Boeuf G, Maxime V, Peyraud C (1996) Effects of growth hormone treatment on oxygen consumption and sea water adaptability in Atlantic salmon parr and pre-smolts Aquaculture 148: 49-62

Seidelin M, Madsen SS, Byrialsen A, Kristiansen K (1999) Effects of insulin-like growth factor-I and cortisol on Na$^+$,K$^+$-ATPase expression in osmoregulatory tissues of brown trout (*Salmo trutta*). Gen Comp Endocrinol 113: 331-342

Sheridan MA (1986) Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen Comp Endocrinol 64: 220-238

Sheridan MA (1989) Alterations in lipid metabolism accompanying smoltification and seawater adaptation of salmonid fish. Aquaculture 82: 191-203

Sheridan MA (1994) Regulation of lipid metabolism in poikilothermic vertebrates. Comp Biochem Physiol 107: 495-508

Sheridan MA, Woo NYS, Bern HA (1985) Changes in the rates of glycogenesis, glycogenolysis, lipogenesis, and lipolysis in selected tissues of the coho salmon (*Oncorhynchus kisutch*) associated with parr-smolt transformation. J Exp Zool 236: 35-44

Shrimpton JM, Patterson DA, Richards JG, Cooke SJ, Schulte PM, Hinch SG, Farrell AP (2005) Ionoregulatory changes in different populations of maturing sockeye salmon *Oncorhynchus nerka* during ocean and river migration. J Exp Biol 208: 4069-4078

Sigholt T, Asgard T, Staurnes M (1998) Timing of parr-smolt transformation in Atlantic salmon (*Salmo salar*): effects of changes in temperature and photoperiod. Aquaculture 160: 129-144

Singer TD, Koreen M, Clements KM, Semple JW, Schulte PM, Bystriansky JS, Finstad B, Fleming IA, McKinley RS (2002) Seawater tolerance and gene expression in two strains of Atlantic salmon smolts. Can J Fish Aquat Sci 59: 125-135

Skilbrei OT (1988) Growth pattern of pre-smolt Atlantic salmon (*Salmo salar* L.) – The percentile increment method (PIM) as a new method to estimate length-dependent growth. Aquaculture 69: 129-143

Skilbrei OT (1991) Importance of threshold length and photoperiod for the development of bimodal length frequency-distribution in Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 48: 2163-2172

Skilbrei OT, Hansen T, Stefansson SO (1997) Effects of decreases in photoperiod on growth and bimodality in Atlantic salmon *Salmo salar* L. Aquaculture Research 28: 43-49

Soivio A, Virtanen E, Muona M (1988) Desmoltification of heat-accelerated Baltic salmon (*Salmo salar*) in brackish water. Aquaculture 71: 89-97

Solbakken VA, Hansen T, Stefansson SO (1994) Effects of photoperiod and temperature on growth and parr smolt transformation in Atlantic salmon (*Salmo salar*) and subsequent performance in seawater. Aquaculture 121: 13-27

Specker JL, Eales JG, Tagawa M, Tyler WA (2000) Parr-smolt transformation in Atlantic salmon: thyroid hormone deiodination in liver and brain and endocrine correlates of change in rheotactic behaviour. Can J Zool 78: 696-705

Staley KB, Ewing RD (1992) Purine levels in the skin of juvenile coho salmon (*Oncorhynchus kisutch*) during parr-smolt transformation and adaptation to seawater. Comp Biochem Physiol 101: 447-452

Staurnes M, Lysfjord G, Berg OK (1992) Parr-smolt transformation of a nonanadromous population of Atlantic salmon (*Salmo salar*) in Norway. Can J Zool 70: 197-199

Staurnes M, Blix P, Reite OB (1993) Effects of acid water and aluminium on parr-smolt transformation and seawater tolerance in Atlantic salmon, *Salmo salar*. Can J Fish Aquat Sci 50: 1816-1827

Staurnes M, Hansen LP, Fugelli K, Haraldstad O (1996) Short-term exposure to acid water impairs osmoregulation, seawater tolerance, and subsequent marine survival of smolts of Atlantic salmon (*Salmo salar* L). Can J Fish Aquat Sci 53: 1695-1704

Stefansson SO, Nævdal G, Hansen T (1989) The influence of three unchanging photoperiods on growth and parr-smolt transformation in Atlantic salmon, *Salmo salar* L. J Fish Biol 35: 237-247

Stefansson SO, Nævdal G, Hansen T (1990) Growth of different families of Atlantic salmon (*Salmo salar*) under three experimental photoperiods. Aquaculture 86: 271-281

Stefansson SO, Björnsson BT, Hansen T, Haux C, Taranger GL, Saunders RL (1991) Growth, parr-smolt transformation, and changes in growth hormone of Atlantic salmon reared under different photoperiods. Can J Fish Aquat Sci 48: 2100-2108

Stefansson SO, Berge AI, Gunnarsson GS (1998) Changes in seawater tolerance and gill Na^+,K^+-ATPase activity during desmoltification in Atlantic salmon kept in freshwater at different temperatures. Aquaculture 168: 271-277

Stefansson SO, Björnsson BT, Sundell K, Nyhammer G, McCormick SD (2003a) Physiological characteristics of wild Atlantic salmon post-smolts during estuarine and coastal migration. J Fish Biol 63: 942-955

Stefansson SO, McGinnity P, Bjornsson BT, Schreck CB, McCormick SD (2003b) The importance of smolt development to salmon conservation, culture, and management: perspectives from the 6th International Workshop on Salmonid Smoltification. Aquaculture 222: 1-14

Stefansson SO, Nilsen TO, Ebbesson LOE, Wargelius A, Madsen SS, Björnsson BT, McCormick SD (2007) Molecular mechanisms of continuous light inhibition of Atlantic salmon parr-smolt transformation. Aquaculture (in press)

Sundell K, Jutfelt F, Agustsson T, Olsen RE, Sandblom E, Hansen T, Bjornsson BT (2003) Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr-smolt transformation of Atlantic salmon, *Salmo salar*. Aquaculture 222: 265-285

Thorpe JE (1989) Developmental variation in salmonid populations. J Fish Biol 35: 295-303

Thorpe JE (1994) Performance thresholds and life-history flexibility in salmonids. Conservation Biol 8: 877-879

Tocher DR, Bell JG, Dick JR, Henderson RJ, McGhee F, Mitchell D, Morris PC (2000) Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils. Fish Physiol Biochem 23: 59-73

Tocher DR, Bell JG, McGhee F, Dick JR, Fonseca-Madrigal J (2003) Effects of dietary lipid level and vegetable oil on fatty acid metabolism in Atlantic salmon (*Salmo salar* L.) over the whole production cycle. Fish Physiol Biochem 29: 193-209

Usher ML, Talbot C, Eddy FB (1988) Drinking in Atlantic salmon smolts transferred to seawater and the relationship between drinking and feeding. Aquaculture 73: 237-246

Utrilla CG, Lobon-Cervia J (1999) Life-history patterns in a southern population of Atlantic salmon. J Fish Biol 55: 68-83

Veillette PA, Young G (2004) Temporal changes in intestinal Na^+, K^+-ATPase activity and in vitro responsiveness to cortisol in juvenile chinook salmon. Comp Biochem Physiol 138: 297-303

Veillette PA, White RJ, Specker JL (1993) Changes in intestinal fluid transport in Atlantic salmon (*Salmo salar* L.) during parr-smolt transformation. Fish Physiol Biochem 12: 193–202

Veillette PA, Sundell K, Specker JL (1995) Cortisol mediates the increase in intestinal fluid absorption in Atlantic salmon during parr-smolt transformation. Gen Comp Endocrinol 97: 250–258

Vijayan MM, Takemura A and Mommsen TP (2001) Estradiol impairs hyposmoregulatory capacity in the euryhaline tilapia, *Oreochromis mossambicus*. Am J Physiol 281: R1161-R1168

Virtanen E, Forsman L (1987) Physiological responses to continuous swimming in wild salmon (*Salmo salar* L.) parr and smolt. Fish Physiol Biochem 4: 157-163

Wargelius A, Fjelldal P-G, Benedet S, Hansen T, Björnsson BT, Nordgarden U (2005) An increase in vertebral GHR and plasma GH/IGF-I is associated with vertebral growth activation, while the upregulation of IGF-IR is related to an increase in vertebral bone density in Atlantic salmon. Gen Comp Endocrinol 142: 163-168

Waring CP, Moore A (2004) The effect of atrazine on Atlantic salmon (*Salmo salar*) smolts in fresh water and after sea water transfer. Aquat Toxicol 66: 93-104

Wedemeyer GA, Saunders RL, Clarke WC (1980) Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar Fish Rev 42: 1-14

Wendt CAG, Saunders RL (1973) Changes in carbohydrate metabolism in young Atlantic salmon in response to various forms of stress. Int Atl Salmon Found, Spec Publ Ser 4: 55-82

Wong CKC, Chan DKO (1999) Chloride cell subtypes in the gill epithelium of Japanese eel *Anguilla japonica*. Am J Physiol 46: R517–R522

Zaugg WS (1981) Advanced photoperiod and water temperature effects on gill Na^+, K^+-adenosine-triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Can J Fish Aquat Sci 38: 758-764

Zaugg WS, Wagner HH (1973) Gill ATPase activity related to parr-smolt transformation and migration in Steelhead trout (*Salmo gairdneri*) – influence of photoperiod and temperature. Comp Biochem Physiol 45: 955-965

Zaugg WS, Adams BL, McClain LR (1972) Steelhead migration – potential temperature effects as indicated by adenosine-triphosphatase activities. Science 176: 415-416

Zheng XZ, Tocher DR, Dickson CA, Bell JG, Teale AJ (2004) Effects of diets containing vegetable oil on expression of genes involved in highly unsaturated fatty acid biosynthesis in liver of Atlantic salmon (*Salmo salar*). Aquaculture 236: 467-483

Zydlewski GB, Haro A, McCormick SD (2005) Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behaviour of Atlantic salmon (*Salmo salar*) smolts. Can J Fish Aquat Sci 62: 68-78

Zydlewski J, McCormick SD (2001) Developmental and environmental regulation of chloride cells in young American shad, *Alosa sapidissima*. J Exp Zool 290: 73-87