
SMOLT PHYSIOLOGY AND ENDOCRINOLOGY

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The parr–smolt transformation of anadromous salmonids is a suite of behavioral, morphological, and physiological changes that are preparatory for downstream migration and seawater entry. The timing of smolt development varies among species, occurring soon after hatching in pink and chum salmon and after one to several years in Atlantic salmon. In many species the transformation is size dependent and occurs in spring, mediated through photoperiod and temperature cues. Smolt development is stimulated by several hormones including growth hormone, insulin-like growth

factor-1, cortisol, and thyroid hormones, whereas prolactin is generally inhibitory. Increased salinity tolerance is one of the most important and tractable changes, and is caused by alteration in the function of the major osmoregulatory organs, the gill, gut, and kidney. Increased abundance of specific ion transporters (Na^+/K^+ -ATPase, $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter and apical Cl^- channel) in gill ionocytes results in increased salt secretory capacity, increased growth and swimming performance in seawater, and higher marine survival.

1. INTRODUCTION

After spending up to several years in freshwater (FW), often in a small territory within a single stream, juvenile salmon abandon their FW life in favor of a long downstream migration followed by an even longer ocean journey. This journey and its accompanying changes have fascinated biologists for centuries. The developmental shift from the stream-dwelling parr to the downstream migrating smolt is known as the parr–smolt transformation, smolting, or smoltification. This transformation includes changes in physiology, behavior, and morphology that are adaptive for downstream migration and an ocean existence. Although not a true metamorphosis, smolting shares the feature of being a radical alteration in development that accompanies a dramatic niche shift.

Smolt development has received substantial research attention in the past 30 years. This research initially was directed at understanding smolt development as it related to the developing aquaculture industry, and in particular the need to control the timing and quality of smolt development for transfer of juveniles into ocean net pens. More recently, the increasing threats to many salmon populations coupled with a realization of the importance of smolt recruitment to natural salmon populations has led to interest in understanding the “natural” biology of smolts and their sensitivity to environmental disturbance (McCormick et al., 2009a). In addition to the influential reviews by William Hoar (1976, 1988), several excellent reviews of smolt biology have appeared (Boeuf, 1993; McCormick et al., 1998; Stefansson et al., 2008). Rather than go over well-tilled ground, wherever possible this review will emphasize new research findings and synthesize the existing literature.

There is large variation in the timing of ocean entry both among and within salmonid species, with some salmon such as pink and chum (*Oncorhynchus gorbuscha*, *O. keta*) entering seawater (SW) soon after

hatching, whereas others such as coho (*O. kisutch*), steelhead trout (*O. mykiss*), and Atlantic salmon (*Salmo salar*) may spend one to several years in FW. In Atlantic salmon, fishes that do not reach the minimum size for smolt development in their first year will wait one year (or more) to smolt, allowing for direct comparison of these two life stages. Although this review will emphasize common elements of smolt development among salmonids, it should be noted that important aspects of timing and environmental effectors differ among salmon species or even within a species; these will be discussed in detail in Section 7.

2. MORPHOLOGY

The morphological differences between parr and smolt can be striking (Fig. 5.1). Parr usually have strong vertical bands, known as parr marks, and spotting that varies among species. During smolting parr marks are reduced and there is development of extensive silvering and darkened fin margins, especially on the caudal, dorsal, and pectoral fins. Silvering is the result of increased deposition of the crystalline purines guanine and hypoxanthine in skin and scales (Johnston and Eales, 1967). Darkened fin margins develop gradually over a period of several weeks owing to the expansion of melanophores (Mizuno et al., 2004).

Smolts are slimmer than parr, and this appears to be due to an increased rate of linear relative to mass growth. This relative slimness is manifested in



Fig. 5.1. Morphological differences between Atlantic salmon parr (top) and smolt reared in the wild. Note the vertical bands and spots on the sides of parr, and the presence of intense silvering and darkened caudal, pectoral, and dorsal fin margins in smolt. Photo credit: S.D. McCormick.

decreased condition factor (weight to length ratio) of smolts during spring, and contrasts with parr which generally increase condition factor in late spring. The reduced condition factor of smolts may in part be due to the energetic demands of smolt development, which include increased basal metabolic rate, increased activity, and reduced lipid content (McCormick and Saunders, 1987). It is also possible that the rapid growth in length allows for greater overall swimming capacity and increased predator avoidance during migration and ocean entry. Shape change analysis has revealed clear differences during smolt development (Beeman et al., 1994) that may be a consequence of the altered pattern of linear and mass growth or perhaps more complex processes.

In addition to these obvious morphological changes, several more subtle changes have been observed. Coho salmon (*O. kisutch*) smolts develop teeth on the maxilla, mandible, and tongue, exhibit increased size of the integumentary folds adjacent to the cloacal opening, and develop a larger auxiliary appendage of the pelvic fin (Gorbman et al., 1982). The relative length of pectoral, pelvic, and anal fins (but not dorsal or caudal) is smaller in Atlantic salmon smolts than in parr (Pelis and McCormick, 2003). Surprisingly, there have been no other morphological analyses to determine whether these or other changes are common to all smolting salmonids.

3. MIGRATION

Downstream migration and movement into the ocean is a critical part of the anadromous life history and thus elemental to smolt biology. A number of behavioral changes occur together as part of downstream migration of smolts, including increased negative rheotaxis to flow (i.e. downstream orientation), decreased agonistic and territorial behavior, and increased schooling and salinity preference (Hoar, 1988; Iwata, 1995). There appears to be a link between altered behavior and the physiological changes that occur during smolting. Gibson (1983) found that Atlantic salmon parr and smolts in artificial streams had similar agonistic behaviors that did not change in spring, and concluded that smolt-related increases in buoyancy and seasonal increases in water velocity were necessary to cause decreased aggression and downstream migration. This finding, along with others, indicates that physiological changes precede and are a requirement for subsequent changes in downstream migratory behavior. Baggerman (1960) suggested that “migration occurs only when the animals are in the proper physiological condition (migration disposition), and at the same time under the influence of appropriate external stimuli which act as ‘releasers’”. Thus,

there are preparatory changes cued by photoperiod and temperature that result in “migratory readiness” of smolts that are then acted on by environmental factors such as temperature, turbidity, and water flow that act as releasing factors to initiate downstream migration of smolts (McCormick et al., 1998). In addition to these environmental factors, other factors such as social cues (i.e. the presence of other migrants) may play a role in initiating downstream migration (Hansen and Jonsson, 1985). The synchronous nature of smolt migration (often occurring over a 3–6 week period) is due to each individual smolt’s reaction to these releasing factors and may have the adaptive value of overwhelming predators by sheer numbers.

It may be useful to divide smolt migration into four major components: initiation, downstream, estuarine, and ocean migrations. Initiation is the initial departure from the FW rearing area. The factors involved in the initiation of migration are probably the most critical in determining the overall timing of smolt migration. Environmental factors that trigger initiation of migration (such as temperature) may not be the same as those that affect patterns or rates of the other components of smolt migration. For instance, initiation of migration may not be strongly regulated by flow (discharge), whereas rates of downstream migration are likely to be strongly influenced by flow (Sykes and Shrimpton, 2010). Indeed, most studies of smolt migration in the wild are unable to distinguish environmental impacts on initiation and downstream movement, as fish counting stations are often far downstream of rearing locations (which are often unknown), and thus will incorporate both initiation and downstream movement. However, laboratory studies (Zydlewski et al., 2005; Sykes and Shrimpton, 2010) and those in the wild in which counting stations are immediately below rearing habitats should be able to determine factors that affect the initiation of migration. For many smolts the initiation of migration will result in SW entry within days or weeks. However, this pattern is not universal, as some species in long river systems may take many weeks before they enter the marine environment. There is some evidence that in some populations smolt migration (or at least a downstream dispersal) may begin a whole year before actual entry into SW (Rimmer et al., 1983). This diversity may have a genetic origin and thus may be adaptive for the river system in which it occurs.

In addition to these four major components of smolt migration, one could add “termination” (thus initiation, downstream migration, and termination), which is usually an artificial construct that can be observed in fish under laboratory conditions. Smolts appear to maintain downstream migratory behavior for only a limited period, 10–20 days (dependent on temperature) in the case of Atlantic salmon (Zydlewski et al., 2005). Under normal (wild) conditions this timing is likely to be sufficient to allow fish to enter the estuary and begin ocean migration. The initiation and termination

of smolt migration is part of a “smolt window”, a limited period of high SW preparedness, which will be discussed in detail below.

There is now a voluminous literature on smolt migration and an exhaustive review of smolt migratory behavior is beyond the scope of this chapter, although such an undertaking would be a valuable contribution. The influence of specific environmental factors on smolt migration, especially the initiation of migration, will be addressed in Section 7 (Developmental and Environmental Regulation) of this chapter. The downstream migration and early ocean entry is a period of high mortality that can be critical to overall survival and strongly affected by FW, estuarine and coastal environmental conditions (Thorstad et al., 2012). Environmental conditions in streams and rivers such as temperature, migratory delays (Rechisky et al., 2009), and contaminants can greatly influence the marine survival of smolts (McCormick et al., 2009a). The comparative life histories including smolt migration strategies of Atlantic salmon, brown trout, and Arctic char have been reviewed by Klemetsen et al. (2012).

Levings (1994) and Thorpe (1994b) document the highly variable use of estuaries among Atlantic and Pacific salmon. The estuarine residence time of Atlantic salmon is relatively brief, generally lasting only one or two tidal cycles (Hansen and Quinn, 1998). In contrast, some species of Pacific salmon may spend many weeks in estuaries before moving out into the open ocean. Schools of postsmolts in estuaries reside in and are displaced with the surface current, and movement is influenced by the tide and the direction of the water flow. Smolts in the upper estuary move by selective ebb tide transport but often display active swimming in the lower estuary or bays.

Recent advances in tagging technology and extensive array systems such as Pacific Ocean Shelf Tracking (POST), the Ocean Tracking Network (OTN), and the Atlantic Cooperative Telemetry Network (ACT) have led to a greater understanding of the early coastal and ocean migratory behavior and survival of smolts (Welch et al., 2011; Tucker et al., 2012; Thorstad et al., 2012), although much remains to be determined. Physiologists have a substantial role to play in this effort by determining the factors that contribute to growth and survival of smolts and providing physiological and endocrine indicators of health.

Several early studies indicated that smolts have reduced swimming ability relative to parr, which led to the hypothesis that downstream migration may be a passive phenomenon in which smolts are no longer capable of maintaining their position in the face of increasing flow rates that normally occur in spring (Thorpe and Morgan, 1978). More recent studies indicate that swimming speeds of Atlantic salmon smolts are the same as or slightly greater than those of parr when standardized for body length, but substantially greater when expressed in absolute speeds (cm s^{-1}) (Peake

and McKinley, 1998). Purely passive processes are therefore unlikely to be the basis for downstream movement of smolts, and “activation” of migration seems likely. This is further supported by estimates of smolt migration speeds that are greater than average flow speeds.

Several physiological and biochemical changes occur in muscle during smolt development that may relate to migration and swimming capacity. Wild Atlantic salmon smolts exhibit a 70% increase in relative heart mass in spring, a phenomenon that is absent in parr under the same conditions (Leonard and McCormick, 2001). A three-fold increase in white muscle phosphofructokinase (PFK) was also observed in smolts, indicative of an upregulation of glycolytic pathways that could supply energy for sustained or repeated burst swimming. White muscle buffering capacity is greater in masu salmon smolts relative to parr, which may minimize pH disturbances associated with anaerobic metabolism (Ogata et al., 1998). Mizuno et al. (2007) observed lower levels of hematocrit, hemoglobin, and burst swimming speed in hatchery masu salmon compared to wild fish, all of which could be remedied by dietary iron supplementation. While these data suggest that burst swimming ability may be high in smolts, an analysis of changes in burst swimming ability during smolt development or a comparison between parr and smolt has yet to be undertaken.

Salinity preference increases during smolt development and is probably associated with the development of increased salinity tolerance (Iwata, 1995). Swimming and predator avoidance ability have been shown to decrease in the first several days after exposure to SW (Jarvi, 1990; Brauner et al., 1992), and are probably due to osmotic perturbations that occur in this period. These observations underline the importance of the heightened salinity tolerance that normally develops during smolting.

4. IMPRINTING

Adult salmon return to their natal stream with very high fidelity, seldom straying to other river systems, a phenomenon that is largely dependent upon olfactory cues (Hasler and Scholz, 1983). The now classic work of Arthur Hasler used artificial odorants to determine that fish could imprint upon a chemical signature during the final stage of smolt development, that fish exposed to odorants had a long-term memory for these compounds (detected through electroencephalography), and that this memory was used for upstream migration. Shifting the location of the imprinted artificial odorants into different streams would shift the final migration destination of adult salmon. Similarly, fish reared in one water source such as a hatchery and released as

smolts into a different river system will return as adults to the river where they were released as smolts (Ueda, 2011). Dittman et al., (1996) further demonstrated that relatively little imprinting occurred before the parr–smolt transformation, and that the migration process itself appeared to be critical for full imprinting to occur. It has been suggested that imprinting may be even more complex than just memorizing the odor of a single location, and that smolts may memorize a series of odorant “waypoints” during their downstream migration and ocean entry (Quinn et al., 1989). Given the diversity of migratory strategies in salmon, it seems likely that some imprinting occurs outside smolting, especially in those populations that begin downstream migration long before SW entry. Extensive reviews of the timing, cues, and mechanisms involved in imprinting can be found in Dittman and Quinn (1996) and Ueda (2011).

4.1. Chemical Nature of Imprinting Odorants

The olfactory system of salmon consists of the sensory olfactory rosettes located in the nares, the olfactory nerve, and the olfactory bulb located in the forebrain. Early studies on electrical responses of the olfactory bulb to exposure of the olfactory rosettes indicated that salmon respond strongly to natal water sources (Ueda, 2011). The compounds inducing these responses were heat stable, dialyzable, and non-volatile. Hara (1994) concluded that, compared to terrestrial animals, salmon can detect only a limited number of chemicals, which largely consist of amino acids, steroids, bile acids, and prostaglandins.

Studies by Hiroshi Ueda and colleagues have determined that amino acids are the most likely sources of the olfactory cues used by salmon in their homing migration. They demonstrated that streams have large natural variation in their composition of specific amino acids, bile acids, and salts. Using olfactory nerve responses as an endpoint, masu salmon were shown to be responsive to the stream water in which they were reared, but not to water from neighboring streams (Shoji et al., 2000). Furthermore, this response could be mimicked by an artificial mixture of amino acids characteristic of their home stream, but not by an artificial mixture of bile acids or salts. Further studies have demonstrated that during their return migration adult male chum salmon are strongly attracted to artificial amino acid mixtures that mimicked those of the stream in which they were reared (Shoji et al., 2003).

Nordeng (1977) proposed that Atlantic salmon and Arctic char (*Salvelinus alpinus*) were imprinting on juveniles of the same species, and potentially those that were most closely related. This process could not apply to all salmon, as juveniles of pink, chum, and ocean-type Chinook salmon are not present in streams when adults are returning. Experiments

with artificial odorants noted above indicate that the presence of juveniles is not necessary for precise homing. While there is evidence for adult sea lamprey (*Petromyzon marinus*) using unique bile acids secreted by conspecific juveniles as attractants (Li et al., 1995), experimental evidence in Pacific and Atlantic salmon does not provide support for conspecific attractants (Brannon and Quinn, 1990). Although it cannot be ruled out that juvenile salmon contribute to the odorants used for imprinting, the weight of evidence indicates that salmon do not use pheromones for homing and that natural amino acids are the primary olfactory cue for the final stage of adult homing in salmon (Brannon and Quinn, 1990; Ueda, 2011).

4.2. Mechanism of Imprinting

Some of the mechanisms involved in olfactory imprinting have been determined by examining changes during smolt development, often in the presence of exposure to natural and artificial odorants. Atlantic salmon smolts have an increased number of filaments and more developed lamellae in the olfactory rosettes compared to parr (Bertmar, 1983). In Chinook salmon (*O. tshawytscha*) there is a rapid increase in “organizational and structural maturity” of the olfactory bulb early in development, and while there is no obvious change associated with smolting, the volume of the olfactory bulb relative to other parts of the brain undergoes a continuous increase from smolting through the adult stage (Jarrard, 1997). Two peaks of olfactory sensitivity and odor learning were detected in Atlantic salmon smolts, one with low response threshold early in smolt development and a second with greater threshold at the peak of smolting (Morin and Doving, 1992). Sockeye salmon (*O. nerka*) can be imprinted on a single amino acid during smolt development (March to June) but not immediately following (July), and a period of 8–14 days was required for imprint memory to be retained (Yamamoto et al., 2010).

There is evidence that some aspects of olfactory imprinting memory are due to retained changes in the olfactory rosettes. Olfactory epithelium of coho salmon imprinted with an artificial odorant during smolting had increased sensitivity to the odorant a year later compared to odorant-naïve fish (Nevitt et al., 1994). Using a similar approach, Dittman et al. (1997) demonstrated that imprinting to an artificial odorant during smolting resulted in greater activity of the second messenger guanylyl cyclase in olfactory cilia following exposure to an artificial odorant, an effect that is only detectable 2 years after imprinting when upstream migration occurs. Altered sensitivity and peripheral memory may also be driven by changes in specific olfactory receptors; in coho salmon the basic amino acid receptor increases during smolting, decreases in postsmolts and then increases again

as adults (A. Dittman, personal communication). The xenobiotic-metabolizing enzyme glutathione-*S*-transferase is located in olfactory receptor cells and is present at higher levels in FW juveniles and returning adult kokanee salmon (*O. nerka*) than it is in subadults in SW (Shimizu et al., 1993). The period of highest responsiveness of sockeye salmon to single amino acids described in the previous paragraph is correlated with the messenger RNA (mRNA) levels of the salmon olfactory-imprinting gene, whose function is currently unknown but which is in a class of compounds involved in olfactory neuron signaling.

It also seems likely that there are “olfactory memories” established in the olfactory bulb during smolting, although the associated mechanisms have not been established. Blood oxygen level-dependent magnetic resonance imaging has revealed that strong responses of adult sockeye salmon to natal stream water occurred in the lateral area of the dorsal telencephalon (Bandoh et al., 2011). The observation that animals become more responsive to previously imprinted olfactory cues during sexual maturation as noted above suggests that the recognition and/or responsiveness to olfactory memory are increased in association with upstream migration and possibly signaled by reproductive hormones.

Recent advances in molecular methods have led to an increasing list of candidate genes that may be involved in imprinting. Transcription of an olfactory receptor gene (SORB) and two vomeronasal receptors (SVRA and SVRC) was found to transiently increase during smolt development of Atlantic salmon (Dukes et al., 2004). Using a microarray approach, Johnstone et al. (2011) found seven members of the OlfC gene family that are putative olfactory receptors have differential transcription levels between juveniles (parr and smolts) and returning adult anadromous Atlantic salmon. No differences in parr and smolt were detected, however, and there were no life history stage differences within a non-anadromous population of the same species. Comparison of Atlantic salmon parr and smolts sampled in spring found 88 genes that were differentially expressed in olfactory rosettes by at least 1.2-fold (Robertson and McCormick, 2012). Upregulated genes that may be specifically involved in olfactory detection and imprinting include olfactomedin, rhodopsin, crystallins, and ubiquitin and ubiquitin-like protein. Olfactomedin is an extracellular matrix glycoprotein that is specifically expressed in the mucus of olfactory neuroepithelium. Rhodopsin and crystallins are involved in eye development, but crystallin genes are expressed in a variety of non-lens tissues and may be involved in neuron growth, and rhodopsin belongs to a family of G-protein coupled receptors that includes the olfactory receptors. Ubiquitin is involved in protein recycling and its increased transcription may be related to cell turnover and/or cell proliferation in the olfactory rosette during

smolting. It should be noted that annotated olfactory receptor genes are not well represented on the GRASP 16K microarray chip used in these studies, and more targeted approaches or larger microarrays will be necessary for a more complete analysis of transcription differences between parr and smolt.

In summary, our current understanding of the mechanisms of imprinting indicates that specific olfactory receptors increase during smolt development, resulting in greater sensitivity of the olfactory epithelium. Exposure to river-specific amino acids during smolting results in formation of a peripheral (rosette-specific) memory, and possibly additional memory formation in the olfactory bulb. These memories are stimulated at the time of upstream migration (possibly by reproductive hormones), leading to high fidelity homing to the natal stream or site of imprinting. Many of the mechanisms and control of these processes have yet to be elucidated.

5. OSMOREGULATION

All teleosts maintain a nearly constant internal osmotic concentration irrespective of their external environment (Edwards and Marshall, 2013, Chapter 1, this volume). The ability to absorb water and secrete salts is therefore critical to the survival of ocean migrating smolts. The capacity for SW osmoregulation increases dramatically during smolt development. Parr generally have only a limited ability to secrete salts, and direct transfer to SW of salinity greater than 30 ppt is usually lethal. Smolts develop increased salinity tolerance over a period of weeks before their entry into SW, the result of coordinated development of the major osmoregulatory organs, the gill, gut, and kidney, as will be described in detail below. These preparatory adaptations for salt secretion result in a high level of salt secretory ability that minimizes osmotic perturbations in SW and allows for rapid movements through estuaries and into full-strength SW.

Increased salinity tolerance is often measured by either increased survival or lower plasma ions and osmolality after direct transfer from FW to high salinity (an SW challenge) (Clarke et al., 1996). Previous work has established that the peak of plasma ion and osmolality changes occurs within 24–48 h after transfer to SW. An example of changes in salinity tolerance can be seen in Fig. 5.2(A); plasma osmolality is high after transfer to 35 ppt early in smolt development (January), but the capacity to maintain low plasma osmolality increases throughout smolt development until it reaches a peak (in mid-May in this case), normally coincident with downstream migration. The levels of plasma osmolality are similar to those seen in FW salmon, indicating that smolts experience almost no osmotic

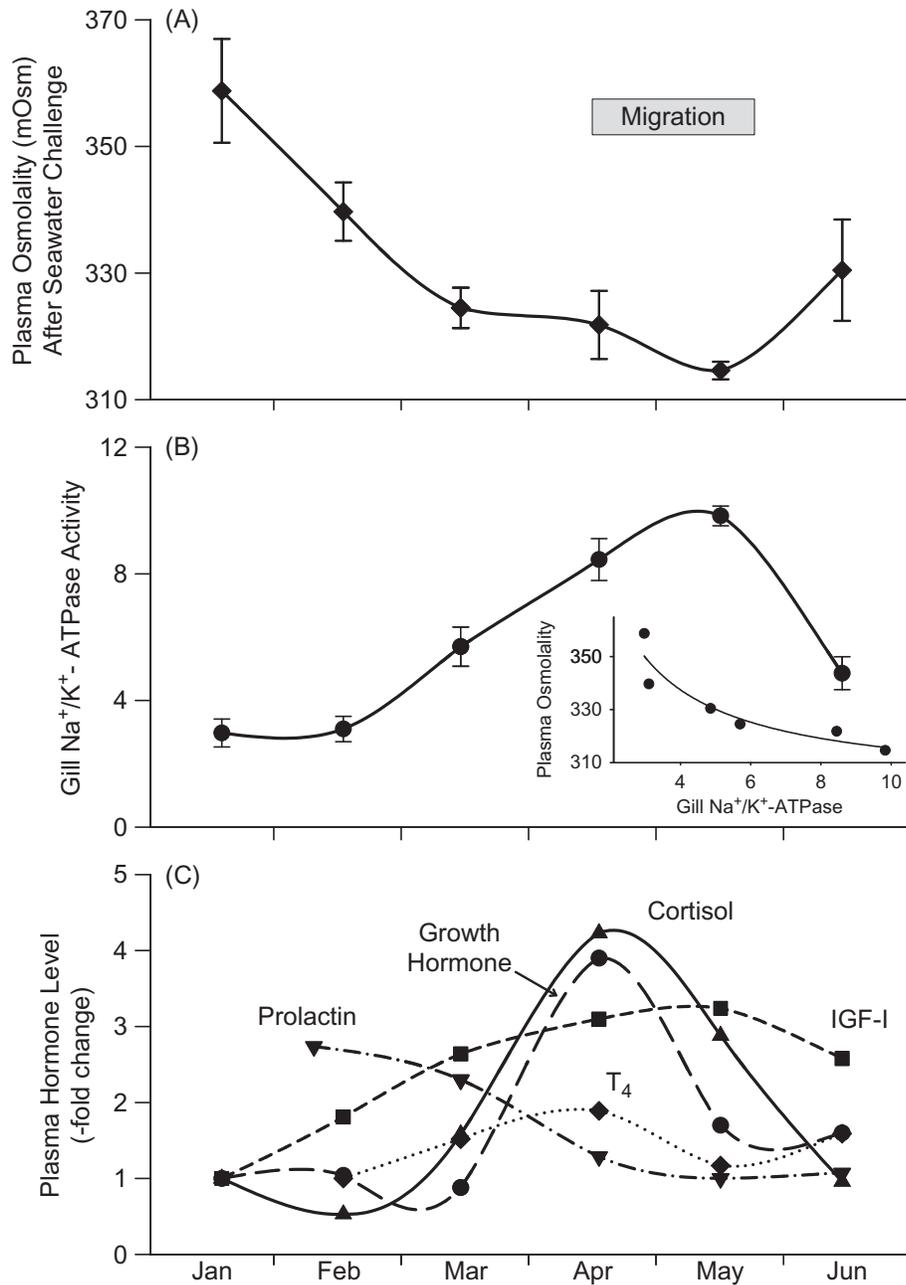


Fig. 5.2. Changes in (A) salinity tolerance, (B) gill NKA activity, and (C) plasma hormone levels during smolt development of Atlantic salmon. Data for plasma hormones are normalized to the changes relative to the first sampling in January and error bars removed for clarity. Salinity tolerance is measured as changes in plasma osmolality 24 h after a direct transfer to 35 ppt seawater. Data are from a single study (McCormick et al., 2009a), with the exception of prolactin (Prunet et al., 1989). NKA: Na^+/K^+ -ATPase; IGF-I: insulin-like growth factor-I; T_4 : thyroxine.

perturbations when transferred at the peak of smolt development. The increase in salinity tolerance that occurs during smolting is also accompanied by an increase in several other metrics of SW performance, including swimming ability, predator avoidance, and growth (McCormick et al., 2009a).

5.1. Gill

The gill is the site of ion uptake in FW and salt secretion in SW that allows euryhaline teleosts to maintain control of their internal salt and water balance (see Evans et al., 2005, for review). Ion transport is primarily carried out by specialized cells in the gill that have been termed ionocytes, chloride cells, or mitochondrion-rich cells. Three major transport proteins are involved in salt secretion and localized to ionocytes. Na^+/K^+ -ATPase (NKA) is located in the basolateral membrane and provides low Na^+ levels and a negative charge within the ionocyte that is used for net Cl^- and Na^+ secretion. The $\text{Na}^+/\text{K}^+/2\text{Cl}^-$ cotransporter (NKCC) is also located in the basolateral membrane and utilizes low Na^+ to transport Cl^- ions into the ionocyte. Chloride can then leave on a “downhill” electrical gradient through an apically located Cl^- channel, the cystic fibrosis transmembrane regulator (CFTR). Sodium leaves the ionocyte through the action of NKA and exits the gill by a paracellular pathway.

It has been known for some time that NKA activity increases in smolts coincident with the development of salinity tolerance (Zaugg and McLain, 1970). The strong positive relationship between gill NKA activity and salinity tolerance (Fig. 5.2) has led to the use of the former as a metric for assessing smolt development and salinity tolerance. Molecular biology studies in rainbow trout (*O. mykiss*) found that there are several isoforms of the catalytic α -subunit of NKA present in the gill, and two of these are differentially regulated by salinity (Richards et al., 2003). Atlantic salmon have two major NKA α isoforms present in distinct ionocytes in the gill: NKA α 1a is most abundant in FW, whereas NKA α 1b predominates in SW (McCormick et al., 2009b). Gill mRNA levels of NKA α 1b increase during smolting of Atlantic salmon, whereas gill NKA α 1a mRNA decreases (Nilsen et al., 2007). Antibodies that are highly specific for each of these salinity-dependent isoforms in Atlantic salmon have recently been developed and have provided new insight into the reorganization of the gill during smolt development (Fig. 5.3; McCormick et al., 2012b). NKA α 1a predominates in FW, and is present in both filamental and lamellar ionocytes. NKA α 1b is present at low levels in FW and is localized to small filamental ionocytes that appear to be below pavement cells and thus not in contact with the external environment. The increase in salinity

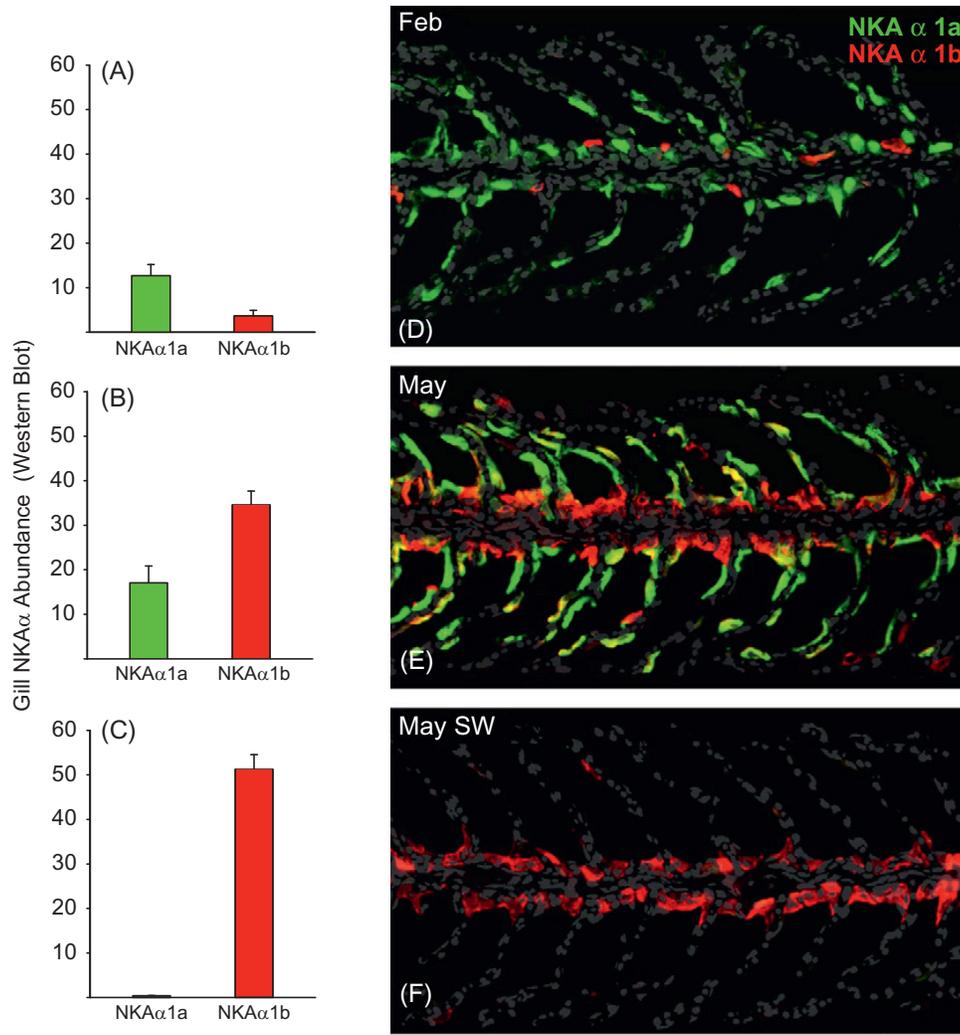


Fig. 5.3. Changes in branchial protein abundance (A–C) and immunolocalization (D–F) of NKAα1a (green) and NKAα1b (red) during smolt development and seawater (SW) exposure of Atlantic salmon (McCormick et al., 2013a). Fish were sampled in freshwater (FW) in February (A,D), FW at the peak of smolting in May (B,E), and after 2 weeks in SW in mid-May (C,F). NKA: Na⁺/K⁺-ATPase.

tolerance in smolts is accompanied by large increases in NKAα1b abundance (10-fold) and cell numbers. A large number of ionocytes with both NKAα1a and NKAα1b are present in smolts in FW, suggesting that NKAα1a ionocytes are gradually transforming to NKAα1b ionocytes. After exposure to SW NKAα1b abundance increases further, there are almost no ionocytes expressing both isoforms, and only a small number of NKAα1a ionocytes remain. These results indicate that increased NKAα1b develops coincident with and is likely to be causal to increased salinity

tolerance of smolts. Gill NKA α 1a protein abundance does not change during smolting but is slightly lower in smolts than in parr. FXYD-11, a potential regulator of NKA activity, is present in ionocytes and increases during smolt development and after SW acclimation (Tipsmark et al., 2010a).

There are two major isoforms of NKCC in vertebrates: the secretory NKCC1 isoform that is present on the basolateral membrane, and the absorptive NKCC2 isoform present on the apical surface. As might be expected from the secretory function of the gill, the prevailing evidence is that an NKCC1 is present in salt-secreting ionocytes of the gill. Using an antibody that recognizes both isoforms, Pelis et al. (2001) observed that the abundance of NKCC and NKCC-positive ionocytes increases during smolt development. Ultrastructural localization of the NKCC to the basolateral membrane suggested that this was the NKCC1 isoform. Partial cloning of a NKCC in Atlantic salmon gill found high sequence similarity with other vertebrate NKCC1 isoforms (>60%) and lower similarity to NKCC2 (<40%), and the mRNA levels of this putative NKCC1 isoform increased five-fold during smolt development (Nilsen et al., 2007). Using an isoform-specific antibody, Christensen and McCormick (unpublished results) found that the NKCC1 is expressed specifically in the gill (and not the intestine), is present primarily in ionocytes, and increases during smolt development. Gill NKCC transcription and abundance are stimulated under normal daylength but not under continuous light; the latter treatment also inhibits development of salinity tolerance, providing further evidence of the importance of NKCC to SW tolerance (Stefansson et al., 2007).

Information on the apical CFTR Cl⁻ channel in smolts comes primarily from transcription studies. There are at least two isoforms of CFTR expressed in the gill. CFTR I mRNA increases substantially during Atlantic salmon smolt development, whereas CFTR II does not change (Nilsen et al., 2007). After SW exposure CFTR I mRNA increased steadily over 2 weeks, whereas CFTR II increased only transiently (Singer et al., 2002). These results suggest that CFTR I is the major gill CFTR isoform associated with increased salt secretory capacity of smolts, although further work on protein abundance and immunolocalization is warranted. Although the full sequence of CFTR is known, production of homologous antibodies has not yielded antibodies with expected distribution patterns on the apical membrane of ionocytes (Regish and McCormick, unpublished results). However, recent screening of several antibodies derived from mammalian CFTR has found one which shows clear apical staining in SW ionocytes but not FW ionocytes of Atlantic salmon (Christensen, Regish, and McCormick, unpublished results). During smolt development there is a large increase in the number of CFTR-positive ionocytes, indicating that all three

major transporters are present in gill ionocytes of smolts before exposure to SW.

The secretion of Na^+ through a paracellular pathway between ionocytes and adjacent cells suggests that intercellular junctions are important for the development of salt secretory capacity. Claudins are a family of membrane proteins that form tight junctions and thus determine transepithelial resistance and ion permeability. [Tipsmark et al. \(2008\)](#) have shown that there are five major claudins expressed in the gills of Atlantic salmon. Claudin 10e mRNA levels increase during smolt development and after exposure to SW, suggesting their involvement in ion secretion. Although the other isoforms did not change during smolt development, claudins 27a and 30 decrease after exposure to SW, suggesting that they are involved in ion uptake.

Aquaporins are a family of proteins involved in cellular and transcellular water movement in vertebrates. Their role in osmoregulation in the gill is not entirely clear as water permeability does not appear to differ substantially in FW and SW in salmonids. However, aquaporins may have a role in regulatory volume decrease or other signaling processes in ionocytes and other cell types in the gill. Three aquaporins have been found to be expressed in the gill of Atlantic salmon ([Tipsmark et al., 2010c](#)). An observed increase in aquaporin (AQP)1b and decrease in AQP3 transcription during smolting and after SW acclimation suggests a physiological role in salt secretion and ion uptake, respectively.

Increased numbers of gill ionocytes during smolt development have been observed in coho and Atlantic salmon ([Richman et al., 1987](#); [Lubin et al., 1989](#)). Ionocytes are the site of both ion uptake in FW and secretion in SW, and most previous histological studies have been unable to distinguish between these functional forms of ionocytes. This is an important knowledge gap as ion uptake demands may also be changing in relation to or independent of the increased salinity tolerance of smolts. [Pisam et al. \(1988\)](#) found that while the total number of ionocytes did not change during smolting of Atlantic salmon, the number of large ionocytes with a well-developed tubular system and association with accessory cells increased in FW during smolt development while the number of small ionocytes with paler mitochondria decreased. Furthermore, there was an increase in the number of NKCC-positive ionocytes during smolt development of Atlantic salmon, which was interpreted as an indicator of increased salt secretory capacity ([Pelis et al., 2001](#)).

The capacity to visualize the abundance and location of the FW and SW isoforms of the NKA α -subunit has provided a useful tool for examining functionally distinct ionocytes during smolt development ([McCormick et al., 2013a](#)). A hypothetical model of the development of SW ionocytes during

smolting is presented in Fig. 5.4. During smolt development there is an increase in the number of $\text{NKA}\alpha 1\text{b}$ ionocytes that also contain NKCC, CFTR, and other functional attributes of salt secretory ionocytes. Based on the colocalization of $\text{NKA}\alpha 1\text{a}$ and $\text{NKA}\alpha 1\text{b}$ in many ionocytes during smolt development and only a small increase in the total number of ionocytes, it may be hypothesized that $\text{NKA}\alpha 1\text{a}$ ionocytes gradually transform to $\text{NKA}\alpha 1\text{b}$ ionocytes. $\text{NKA}\alpha 1\text{b}$ ionocytes of smolts in FW probably lie quiescent below the surface of the gill filament until they are rapidly activated by exposure to SW, thus providing the high salt secretory capacity and salinity tolerance that is characteristic of smolts. The rapid exposure of ionocytes is supported by the observation that the number of ionocytes increases during smolt development of Atlantic salmon but the number of surface-exposed ionocytes does not change until SW exposure, increasing within 1 day and continuing through 5 days (Lubin et al., 1989). Further increases in $\text{NKA}\alpha 1\text{b}$ cell size and abundance after exposure to SW presumably confer on smolts an even greater level of salt secretory capacity, a built-in safety factor to allow for ion transport demands under a wide variety of conditions.

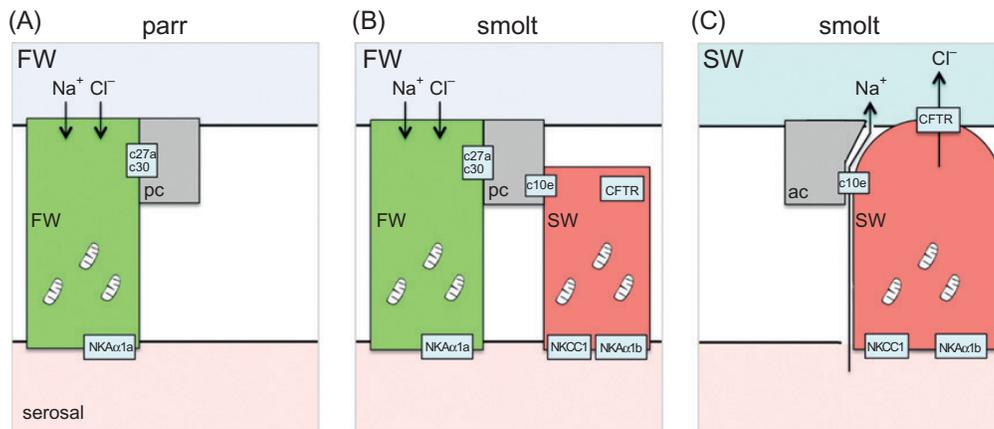


Fig. 5.4. Schematic of gill $\text{NKA}\alpha 1\text{a}$ and $\text{NKA}\alpha 1\text{b}$ ionocyte changes during smolt development in freshwater (FW) and after seawater (SW) exposure. In FW parr (A), $\text{NKA}\alpha 1\text{a}$ ionocytes predominate. During smolt development the numbers of $\text{NKA}\alpha 1\text{b}$ and colabeled cells increase (B). These cells appear to be beneath pavement cells and thus likely to be inactive. After exposure to SW (C), $\text{NKA}\alpha 1\text{b}$ increase in size and now have clear contact with the external environment, while $\text{NKA}\alpha 1\text{a}$ cells disappear. NKCC, CFTR, and claudin 10e are also upregulated in FW during smolt development and increased further after exposure to SW. Claudins 27a and 30 are downregulated during smolt development and exposure to SW. NKA: Na^+/K^+ -ATPase; NKCC: $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter; CFTR: cystic fibrosis transmembrane regulator; ac: accessory cell.

5.2. Gut

In SW teleosts drink more than in FW in order to absorb water lost to the hyperosmotic medium. In order to take up water the gut first absorbs Na^+ and Cl^- to reduce the osmotic pressure of the intestinal fluid. The latter is accomplished by the coordinated action of an apical, absorptive $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter (NKCC2) and a basolateral NKA. Active alkalization further reduces the osmotic pressure of the gut by causing the precipitation of Mg^{2+} , Ca^{2+} , and SO_4^{2-} . Paracellular and transcellular water absorption then takes place, for most species in the anterior intestine (Grossell, 2011; Edwards and Marshall, 2013).

In coho and Atlantic salmon fluid transport (J_v) of the isolated posterior intestine increases during smolting and after SW exposure (Collie and Bern, 1982; Veillette et al., 1993). Both water transport capacity and NKA activity increased in anterior (pyloric ceca) and posterior segments of the intestine of yearling Chinook salmon during early summer just before the development of salinity tolerance (Veillette and Young, 2004). In a smolting population of brown trout (*Salmo trutta*) intestinal water transport capacity increased five-fold but was not accompanied by increased intestinal NKA activity (Nielsen et al., 1999). Sundell et al. (2003) found increased intestinal NKA activity and paracellular permeability (as measured by transepithelial resistance) in association with increased J_v in the anterior intestine of Atlantic salmon smolts. These authors observed a decrease in transepithelial resistance after SW exposure, suggesting a shift from paracellular to transcellular routes of water uptake.

Recent work on gene expression has suggested some of the underlying transporters that may be involved in altered intestinal water transport capacity of smolts. Tipsmark et al. (2010b) observed increased AQP8b mRNA levels during smolting, whereas AQP1a, 1b, and 8b transcription all increased after SW acclimation. Although no changes were detected in intestinal claudins during smolt development, claudins 15 and 25b were upregulated after exposure to SW (Tipsmark et al., 2010b), suggesting their involvement in paracellular permeability that is altered after SW exposure.

5.3. Kidney and Urinary Bladder

The kidney of FW teleosts creates a highly dilute urine to excrete excess water, whereas in SW the urine is isosmotic with plasma but contains excess divalent cations (Edwards and Marshall, 2013, Chapter 1, this volume). Surprisingly little work has been done to quantify renal function or changes in ion transporter abundance or other proteins involved in salt and water transport in the kidney during smolt development. The function of the

kidney is complex and highly regionalized, and there are no well-established biochemical markers for function in FW and SW. Urine production of Atlantic salmon smolts increased in spring coincident with increased gill NKA activity (Eddy and Talbot, 1985). However, kidney NKA activity does not appear to change in Atlantic salmon smolts or juveniles exposed to SW (McCormick et al., 1989).

The urinary bladder also has an important role in ion reabsorption in FW in many teleost species. Sodium and Cl^- reabsorption by the urinary bladder of coho salmon smolts held in FW did not change between March and June (Loretz et al., 1982). When transferred to SW in May, poor survival was accompanied by high levels of urinary bladder Na^+ and Cl^- reabsorption, characteristic of FW fishes and maladaptive for SW. High survival after SW transfer in June was accompanied by low levels of Na^+ and Cl^- reabsorption. Thus, while no obvious functional change had occurred in FW, there was a developmental increase in the ability of the urinary bladder to respond appropriately to SW exposure.

5.4. Is Freshwater Osmoregulation Compromised during Smolting?

The previous sections have shown that the underlying mechanisms of water absorption by the gut and salt secretion by the gill change during smolt development and are associated with a high level of salinity tolerance not present in earlier developmental stages. However, these changes may impair the physiological capacity of smolts to regulate water and salts while they still reside in FW, before and during their seaward migration. While most studies report minimal changes in the levels of the major plasma ions during smolt development (McCormick and Saunders, 1987), smolts appear to be more sensitive to external factors that affect osmoregulation. Identical handling stresses of parr and smolts result in greater loss of plasma ions in the latter (Carey and McCormick, 1998). There is evidence that Atlantic salmon experience altered Na^+ flux levels during smolting (Primmitt et al., 1988), although some or all of these changes may be due to the stress of confinement inherent in these measurements. Atlantic salmon smolts lose ions and have lower survival than other life stages after exposure to acid and aluminum, which negatively affects gill ionocytes and NKA (Rosseland et al., 2001; Monette and McCormick, 2008). Many heavy metals also exert their main toxic effect through their impacts on osmoregulation. Coho salmon smolts in May were found to be more sensitive to zinc and copper than they were as parr in November (Lorz and McPherson, 1976). While the FW NKA α 1a isoform does not change in abundance over the course of smolt development, it is slightly lower in smolts than in parr, and the number of NKA α 1a ionocytes decreases, suggesting a possible decrease in

the capacity for ion uptake (McCormick et al., 2013a). Thus, it would appear that while smolting does not necessarily impair ionoregulatory homeostasis in FW, smolts may be more sensitive to environmental factors that affect ion regulation.

Thorpe (1994a) has argued that smolt development should be viewed as a maladaptation to or abandonment of FW. The recent findings that the major changes in NKA in the gill during smolting are due to increases in the “SW isoform” (NKA α 1b) support a more adaptationist interpretation of smolting; that is, that it is primarily an adaptation for increased osmoregulatory capacity in SW and other adaptive changes for SW performance (McCormick et al., 2013a). Increased sensitivity of smolts to osmoregulatory disturbance probably represents constraints on the overall capacity of osmoregulatory systems.

6. ENDOCRINE CONTROL

Smolting has been described as a pan-hyperendocrine state (Bern, 1978), where many hormones that have different physiological actions are increasing during development, although not necessarily at the same time or rate (Fig. 5.5). This makes smolt development different from metamorphic events where most of the developmental events that occur are often controlled by one stimulatory hormone and possibly one inhibitory hormone. It can be argued that this allows flexibility among the various aspects of development (physiology, morphology, behavior) that occur during smolting. This flexibility may be particularly important to the fitness of populations and individuals, in that behavioral changes such as the initiation of migration may be occurring at different times in relation to other developmental events such as SW entry. However, the extent of population variability in various aspects of smolt development has yet to be established.

6.1. Growth Hormone/Insulin-Like Growth Factor-1

Growth hormone (GH) and insulin-like growth factor (IGF-I) both appear to be central to the smolting process. Increases in circulating levels of GH during smolt development have been shown for Atlantic and coho salmon (Björnsson et al., 2011) (Fig. 5.2C). Increased plasma GH is due to an increase in pituitary secretion early in smolt development and later by increases in GH synthesis and secretion (Agustsson et al., 2001). No change in the number of growth hormone-releasing hormone (GHRH) neurons was observed in the brain of chum salmon during smolting (Parhar and Iwata,

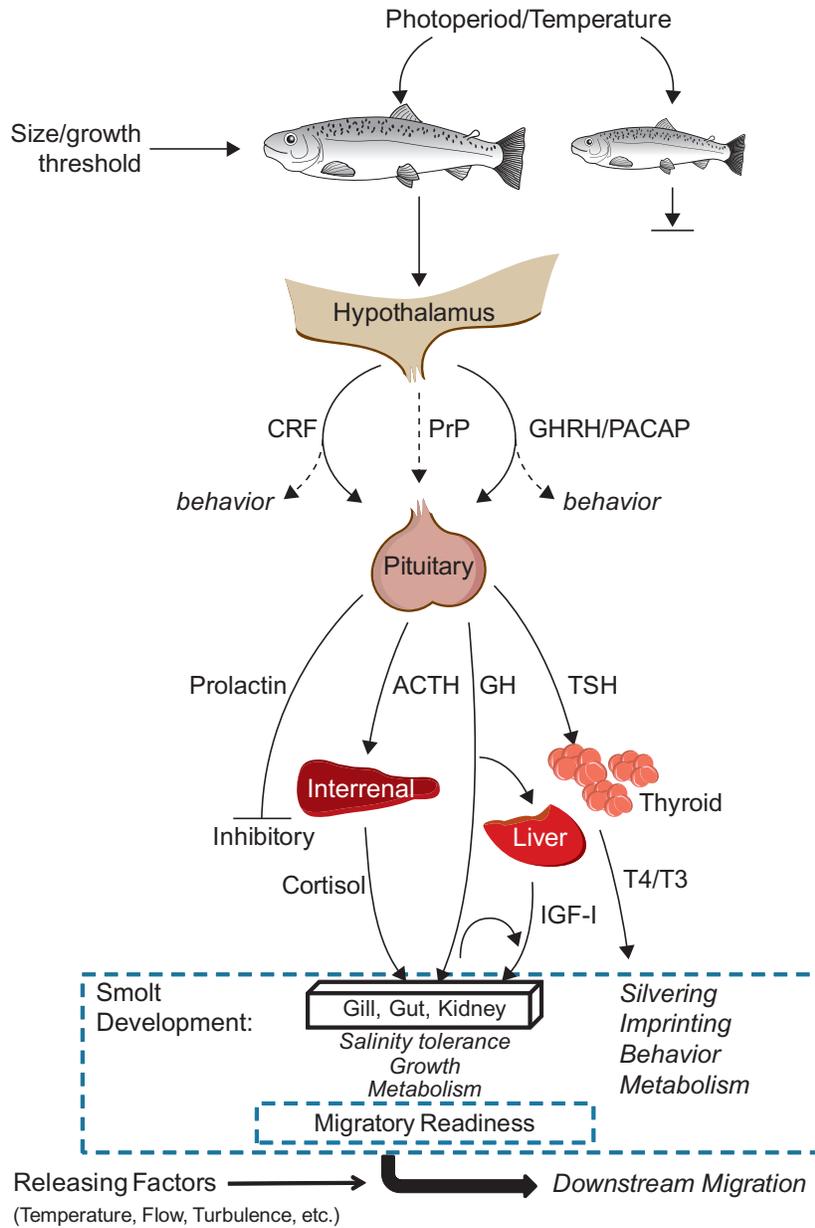


Fig. 5.5. Schematic of the neuroendocrine control of smolt development. A size- or growth-related threshold results in increased response of the light–brain–pituitary axis to stimulate circulating levels of growth hormone (GH), cortisol, and thyroid hormones. GH and cortisol interact to control hyperosmoregulatory mechanisms in the gill, gut, and kidney, resulting in increased salinity tolerance, as well as changes in growth and metabolism. Thyroid hormones have a role in morphological changes such as silvering, imprinting, metabolism, and possibly behavior. Prolactin is generally thought to be inhibitory to most aspects of smolt development. There is also substantial interaction among these endocrine components, including the regulation of hormone secretion and peripheral receptors. It should be noted that early development of salinity tolerance and other aspects of smolting in pink and chum salmon appear to be independent of photoperiod cues and to be driven primarily by developmental processes. CRF: corticotropin-releasing factor; PrP: prolactin-releasing peptide; GHRH: growth hormone-releasing hormone; PACAP: pituitary adenylate cyclase-activating peptide; ACTH: adrenocorticotropic hormone; TSH: thyroid-stimulating hormone.

1996). In Atlantic salmon photoperiod appears to be the major factor regulating increased GH, with temperature affecting the rate of response to photoperiod (McCormick et al., 2000). There is a strong association between increased plasma GH levels and osmoregulatory indicators of smolt development. During spring Atlantic salmon parr have constant, low levels of plasma GH and cortisol; however, both increase dramatically in smolts (McCormick et al., 2007). In landlocked Atlantic salmon in the spring there is no increase in plasma GH at a time when it is elevated in anadromous conspecifics, and this is associated with poor salinity tolerance (Nilsen et al., 2008). GH appears to be the first hormone to increase following photoperiod manipulation and has an important controlling or interaction effect with other endocrine axes during smolting (see below); GH therefore appears to be critical to the initiation and overall timing of smolt development.

Plasma IGF-I also increases during smolting (Fig. 5.2C), although the pattern is usually not identical to that of circulating GH. Although GH is generally recognized as the major secretagogue for plasma IGF-I that is primarily released from the liver, it is clear that other factors are also involved. In Atlantic salmon plasma IGF-I has been shown to increase in both parr and smolts in spring, with smolts exhibiting higher levels throughout (McCormick et al., 2007), but not all studies have found increasing plasma IGF-I during smolting (Nilsen et al., 2008). Increased IGF-I occurs in association with smolting in both spring and autumn in the highly plastic Chinook salmon (Beckman and Dickhoff, 1998). Plasma IGF-I levels and gill NKA activities in spring Chinook salmon smolts reared at different hatcheries were significant predictors of the adult return rates to the hatcheries (Beckman et al., 1999), indicating that environmental conditions acting through smolt developmental pathways are important for marine survival. In addition to changes in circulating levels, local production of IGF-I by target tissues is also likely to be involved in smolt development. Liver and gill IGF-I mRNA increase during smolting of coho salmon (Sakamoto et al., 1995). Increased transcription of both IGF-I and IGF-I receptor in the gill has been found in anadromous Atlantic salmon smolts but is absent in a landlocked strain (Nilsen et al., 2008). Transcription of branchial GH receptor also increases during smolt development of Atlantic salmon (Kiilerich et al., 2007b), which, along with increased circulating GH, may explain the observed increases in branchial production of IGF-I. There is relatively little information on regulation of the GH-IGF-I axis in pink and chum salmon that smolt early in development. This is likely to be due to measurement limitations associated with their small size, although the advent of molecular approaches should make it possible to examine transcriptional changes.

Exogenous treatment with GH and IGF-I can increase the salinity tolerance of juvenile trout and salmon (Fig. 5.5) (see also Takei and McCormick, 2013, Chapter 3, this volume). Increased transcription and/or protein abundance have been observed for the major gill transport proteins involved in ion secretion (NKA α 1b, NKCC1, and CFTR) (Tipsmark and Madsen, 2009; McCormick et al., 2013a), suggesting that GH controls a differentiation program for salt-secreting ionocytes. There is an important interaction between the GH–IGF-I axis and cortisol that will be discussed in detail below.

GH and IGF-I are important regulators of growth and metabolism in teleost fishes (Wood et al., 2005). GH and cortisol are lipolytic in salmon (Sheridan, 1986) and their combined increase is probably responsible for the increased linear growth relative to mass growth (lower condition factor) that occurs during smolt development. Surprisingly, the role of the GH–IGF-I axis in the restoration of condition factor and the increased scope for growth that occurs after smolting has not been examined. Reduced liver GH receptor and IGF-I production accompany the “stunting” phenomenon, where impaired growth is observed in some individuals after SW exposure (Duan et al., 1995). It has been suggested that this occurs when fish are exposed to SW before the development of SW tolerance, although pathways involved in the genesis of this phenomenon have not been established.

There is intriguing evidence that the GH–IGF-I axis is involved in behavioral changes during smolting. GH treatment increases the salinity preference of coho salmon, an effect that is augmented with coincident thyroxine (T₄) treatment (Iwata et al., 1990). Intracerebral treatment with GHRH stimulated both downstream movement and schooling behavior in chum salmon fry, while corticotropin-releasing hormone (CRH), melatonin, and serotonin stimulated downstream movement only (Ojima and Iwata, 2009). Behavioral experience may also feed back onto the GH–IGF-I axis.

There has been increasing emphasis on understanding endocrine changes that occur in wild and hatchery salmon during downstream migration and early ocean entry (Björnsson et al., 2011). Fig. 5.6 presents a compilation of data from two studies on endocrine changes during migration of Atlantic salmon in river, coastal, and ocean environments (Stefansson et al., 2003; McCormick et al., 2013b). The results indicate that salmon smolt plasma GH levels increase in the river and after initial SW entry but in the long term (after several weeks) in the ocean are relatively low. Initial increases in plasma GH levels may be due to the metabolic demands of migration and the response to SW exposure. Since levels of plasma GH are low and IGF-I high in rapidly growing animals, the lower levels of plasma GH and higher IGF-I in the open ocean probably reflect the high growth rate of postsmolts (Stefansson et al., 2003).

6.2. Cortisol

Early histological studies showed hyperactivity of the interrenal cells during smolt development (see [Specker, 1982](#), for review), and circulating cortisol was first shown to increase in Atlantic salmon smolts by [Fontaine and Hatey \(1954\)](#) and later in coho salmon by [Specker and Schreck \(1982\)](#). These results have since been confirmed by a large number of studies, although information in early smolting species, such as chum and pink salmon, is absent. Plasma levels of cortisol remain low and constant in Atlantic salmon parr in spring, but increase 10-fold in smolts held under the same conditions ([McCormick et al., 2007](#)). Similarly, plasma cortisol increases during smolting in anadromous Atlantic salmon, but spring increases are absent in a landlocked strain ([Nilsen et al., 2008](#)).

There is still an incomplete understanding of the factors that regulate the hypothalamic–pituitary–interrenal axis during smolting. The number of CRH neurons increases during smolt development of Atlantic salmon, at least in part due to upregulation by thyroid hormones ([Ebbesson et al., 2011](#)). Surprisingly, there has been no measure of the major cortisol secretagogue, adrenocorticotrophic hormone (ACTH), either in circulation or in the pituitary, during smolt development. A marked increase in the sensitivity of the interrenal to produce cortisol in response to ACTH was observed in April in smolting coho salmon, which occurred several weeks

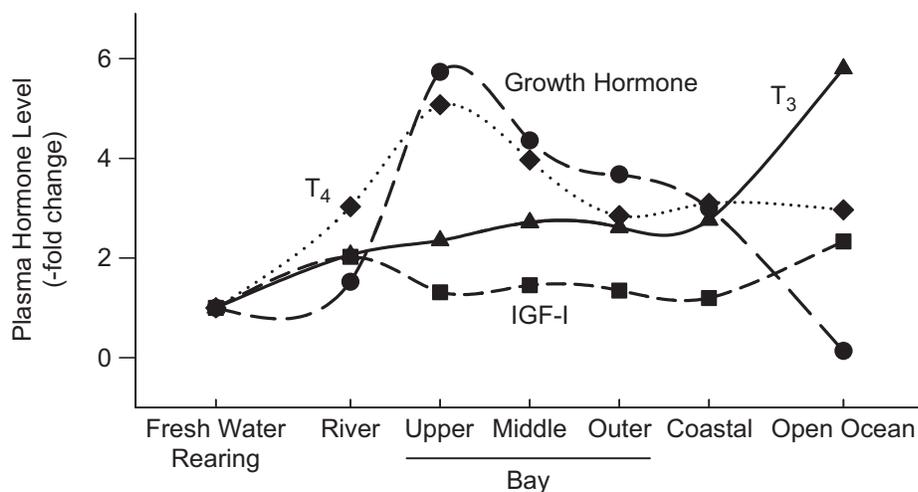


Fig. 5.6. Changes in plasma growth hormone, insulin-like growth factor-1 (IGF-I), thyroxine (T₄), and triiodothyronine (T₃) in Atlantic salmon smolts during freshwater (FW) rearing, river migration, and ocean entry. The graph is a composite from two studies: [McCormick et al. \(2013b\)](#) for river, bay, and coastal results, and [Stefansson et al. \(2012\)](#) for open ocean. Initial FW levels (river levels in the case of [Stefansson et al., 2012](#)) were normalized to 1.0 and other values expressed as a fold-change relative to these values.

before the peak in circulating cortisol (Young, 1989). This enhanced ACTH sensitivity is likely to be due to a direct effect of GH on the interrenal (Young, 1988), which can also explain how GH treatment leads to increased circulating cortisol levels (Quinn et al., 2003). Plasma clearance rate of cortisol increases in late spring and declines in summer in coho salmon smolts in FW (Patino et al., 1985) and after SW transfer (Redding et al., 1984), indicative of increased utilization, metabolism, or clearance of cortisol.

Using classical binding studies, the number of gill cortisol receptors was shown to decrease during smolt development of coho salmon (Shrimpton et al., 1994). This may in part be due to the effect of increasing endogenous cortisol, which decreases the number of ligand-free receptors (Shrimpton and Randall, 1994). In Atlantic salmon, both parr and smolt exhibited large increases in gill cortisol receptors in the spring (Shrimpton and McCormick, 1998b). Immunocytochemical and *in situ* hybridization approaches have shown higher levels of cortisol receptors in ionocytes compared to other cell types in the gill (Uchida et al., 1998). Recent molecular data indicate that there are one or two “glucocorticoid” receptors (GRs) and one “mineralocorticoid” receptor (MR) present in most teleosts; this nomenclature is based on their similarity to mammalian receptors that have distinct regulatory pathways for metabolic and ion regulatory effects of corticosteroids, although such distinctions do not apply to fish (Takei and McCormick, 2013, Chapter 3, this volume). Increased gill GR transcription has been observed in smolting masu and Atlantic salmon, whereas gill MR transcription does not change (Mizuno et al., 2001; Nilsen et al., 2008). Based on the current weight of evidence, it seems likely that one or both GRs signal most of the actions of cortisol to promote salt secretion, and that MR may also play a more limited role in osmoregulation in most species. The high level of MR transcription in the teleost brain suggests a role for this receptor in regulating behavior and/or neuroendocrine responses. Since cortisol appears to have actions in both ion uptake and salt secretion, some of the differential actions of GR and MR may relate to these dual actions of cortisol, although evidence for a role in MR in salmonid osmoregulation is still lacking.

Exogenous treatment with cortisol stimulates salinity tolerance and many of the underlying mechanisms involved in SW osmoregulation in the gill and the gut (McCormick, 2001). The major transport proteins involved in salt secretion, NKA α 1b, NKCC1, and CFTR, can be increased by cortisol treatment, as can the abundance of ionocytes (Pelis and McCormick, 2001; Küllerich et al., 2007a; McCormick et al., 2008). Using NKA activity as an endpoint, *in vitro* responsiveness of coho salmon gill to cortisol was low in winter, increased in spring just before endogenous

increases in gill NKA activity, and subsequently declined in late spring and early summer (McCormick et al., 1991). Cortisol also increases transcription of gill claudin 28e, a tight junction protein that increases after SW acclimation of Atlantic salmon (Tipsmark et al., 2009). Cortisol treatment increases the intestinal water uptake capacity and NKA activity in juvenile Atlantic and Chinook salmon (Veillette et al., 1995; Veillette and Young, 2004).

There is an important interaction between the GH–IGF-I axis and cortisol that underlies the development of hypoosmoregulatory capacity of smolts. As noted above, GH increases the responsiveness of the interrenal to ACTH, increasing the amount of cortisol released at any given level of ACTH. GH also upregulates the abundance of corticosteroid binding sites and transcription of GR in the gill (Shrimpton et al., 1995; Kiilerich et al., 2007b). In turn, cortisol increases the transcription of gill GH and IGF-I receptors (Tipsmark and Madsen, 2009). In Atlantic salmon juveniles, cortisol treatment increases both the FW and SW isoforms of NKA ($\alpha 1a$ and $\alpha 1b$), but when coinjected with GH cortisol decreases NKA $\alpha 1a$ and increases NKA $\alpha 1b$, and results in high salinity tolerance similar to that of smolts (S.D. McCormick, unpublished results). Thus, GH appears to be acting as a switch for the actions of cortisol, moving it away from ion uptake and promoting salt secretion. While this interaction is well established for the gill, there is little evidence for its importance in the gut or kidney. Intestinal claudin 25b transcription is upregulated after SW exposure and GH treatment, but there is no apparent preparatory increase in transcription of this gene during smolting (Tipsmark et al., 2010b).

It seems likely that the hypothalamic–pituitary–interrenal (HPI) axis plays a role in behavioral changes during smolting. Corticotropin-releasing factor (CRF) increased downstream migratory behavior in juvenile coho and chum salmon (Clements and Schreck, 2004; Ojima and Iwata, 2009, 2010). The rapid response to this central administration suggests a direct response to this peptide rather than an action through cortisol, although the latter cannot be ruled out. Cortisol has been shown to increase the salinity preference of juvenile coho salmon, although to a lesser extent than GH or T_4 (Iwata et al., 1990). The HPI axis may also play a role in some of the morphological changes that occur during smolting, since ACTH (but not cortisol) has been found to increase fin darkening in Atlantic salmon (Langdon et al., 1984), a characteristic smolt-related change in this species.

6.3. Prolactin

Histological studies of the pituitary gave the first indication that prolactin played a role in smolt development, with greater secretory

“activity” present in FW smolts than in parr or SW smolts (Nishioka et al., 1982). In both Atlantic and coho salmon, plasma prolactin is elevated in winter and early spring but decreases in April and May when smolting peaks (Fig. 5.2C) (Prunet et al., 1989; Young et al., 1989). Given the negative impacts of prolactin on salt secretory capacity (see below), this decrease can be viewed as removing an inhibitory factor that allows normal progression of smolt development. Although seasonal changes in plasma prolactin were observed in amago salmon (*O. rhodurus*), there were no obvious increases in silvering and salinity tolerance that occur in autumn in this species (Yada et al., 1991). Plasma prolactin is lower in downstream migrating Arctic char than in non-migrants (Hogasen and Prunet, 1997). As in most other teleosts, plasma prolactin levels are strongly reduced after SW exposure, further supporting the idea that prolactin promotes ion uptake and is inhibitory to salt secretion (Prunet et al., 1989; Young et al., 1989; Yada et al., 1991).

Information on the factors involved in prolactin regulation during smolting is limited. A hypothalamic prolactin-releasing peptide has recently been found in vertebrates including trout and salmon (Moriyama et al., 2002), but has not been examined in smolts. Unlike the euryhaline tilapia in which the direct response of prolactin-producing cells in the pituitary to osmolality is well established, the prolactin cells of coho salmon do not alter synthesis or secretion in response to physiological changes in osmolality (Kelley et al., 1990), but do appear to be responsive to calcium (MacDonald and McKeown, 1983). Cortisol directly inhibits prolactin synthesis and release, which may provide a mechanism for decreased prolactin during the peak of smolting and after SW exposure (Kelley et al., 1990). As noted above, cortisol has a role in ion uptake in fish, and there is some indication that prolactin and cortisol interact to promote ion uptake. The mechanism for this interaction has not been established, however, and there is little known of the interaction of cortisol and prolactin during smolt development.

In the euryhaline tilapia (*Oreochromis mossambicus*), prolactin receptors are found at high levels in osmoregulatory organs such as gill and kidney (Dauder et al., 1990). Transcription of gill prolactin receptors remains stable or decreases during smolting of Atlantic salmon, and decreases further after 1 month in SW (Küllerich et al., 2007b; Nilsen et al., 2008). Prolactin receptor abundances in gill, gut, or kidney during smolting have not been examined.

Exogenous treatment with prolactin has been shown to decrease salinity tolerance of smolts (T.O. Nilsen, personal communication) and brown trout in autumn (Seidelin and Madsen, 1997). Prolactin also inhibits some of the mechanisms associated with developmental increases in salt secretion (Fig. 5.5), including GH- and cortisol-induced elevations of gill NKA activity (Seidelin and Madsen, 1997) and SW levels of NKA activity and NKA α 1b transcription (Tipsmark and Madsen, 2009). This effect may occur

primarily through the action of prolactin to decrease the capacity of the gill to respond to IGF-I (Seidelin and Madsen, 1999). Given the observed impact of prolactin on “water drive” behavior in amphibians, it is certainly possible that prolactin has behavioral effects in smolts, but this has yet to be examined.

6.4. Thyroid Hormones

Using a histological approach, Hoar (1939) found evidence for increased “activity” of the thyroid gland during smolt development of Atlantic salmon. The capacity to measure circulating levels of thyroid hormone by radioimmunoassay in salmon, first developed by Dickhoff et al. (1978), has led to a large number of studies that indicate increased circulating levels of T_4 and to a lesser extent triiodo-L-thyronine (T_3) during smolting of Atlantic and Pacific salmon (Fig. 5.2C) (Dickhoff and Sullivan, 1987). Large increases in plasma T_4 have been observed in hatchery smolts after release and in wild smolts during migration, and are possibly related to the stimulation of the thyroid axis by the act of migration itself (Iwata et al., 2003; McCormick et al., 2003). Increased plasma T_4 also occurs after exposure to “novel water” (water with a different chemical composition) (Hoffnagle and Fivizzani, 1990) and may play a role in imprinting, as discussed in detail below. Lunar rhythms in plasma T_4 have also been observed in coho salmon smolts (Grau et al., 1981) and are associated with lunar periodicity of movement into estuaries that has been observed in some populations. There is recent evidence that thyroid hormones increase during entry of smolts into estuarine and near-coastal environments (Fig. 5.6).

In their review of the role of thyroid hormones in smoltification, Dickhoff and Sullivan (1987) emphasized the metabolic and morphological impacts of this endocrine axis. Exogenous thyroid hormones have been shown to increase silvering, but not fin darkening, in several salmonid species. Hemoglobin isoforms change during smolt development and similar changes can be induced by thyroid hormone treatment. The increased lipolysis and reduced lipogenesis that occur during smolting can also be induced by thyroid hormone treatment, although as noted above GH and cortisol appear also to be involved in these metabolic changes.

There is both direct and indirect evidence that thyroid hormones play a role in imprinting. Treatment of presmolt coho salmon with T_4 resulted in the establishment of long-term odor “memories”, whereas untreated fish did not imprint on odors (Hasler and Scholz, 1983). Increased responsiveness of olfactory tissue to amino acids has been found in Atlantic salmon in association with increased plasma T_4 levels (Morin et al., 1994), although paradoxically, T_4 treatment reduces olfactory sensitivity to alanine (Morin

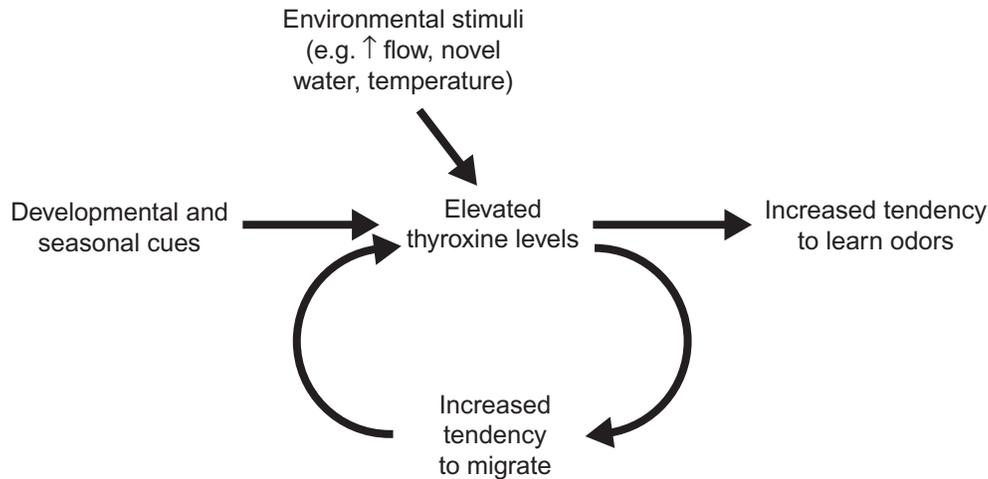


Fig. 5.7. Schematic of impacts smolt development, environmental stimuli, and migration on plasma thyroxine levels and the tendency to learn odors and imprint. From [Dittman and Quinn \(1996\)](#) with permission.

[et al., 1995](#)). Thyroid hormone receptors have been found in the olfactory neurons and olfactory bulb of masu salmon ([Kudo et al., 2002](#)). Increased cell proliferation in olfactory rosettes was detected in coho salmon smolts treated with T_3 ([Lema and Nevitt, 2004](#)). [Dittman and Quinn \(1996\)](#) suggested that thyroid hormone elevations before and during smolt development cue the imprinting of odor memories, and that both the experience of novel water and the act of migration feed back on one another to increase thyroid hormones and promote odor learning ([Fig. 5.7](#)). The observed increase in plasma thyroid hormones during estuarine and near-coastal migration ([Fig. 5.6](#)) suggests that imprinting (olfactory and/or spatial) may occur not only during downstream migration but also during early ocean entry ([Iwata et al., 2003](#); [McCormick et al., 2012](#)).

Thyroid hormones have been shown to have effects on the behavior of fish, and there are several links between thyroid hormones and downstream migratory behavior. Aggressive behavior decreases during smolt development (presumably in order to promote schooling), and thyroid hormone treatment has been shown to decrease aggressive behavior in anadromous, but not in non-anadromous, salmonids ([Hutchison and Iwata, 1998](#)). In his review of the role of thyroid hormones and cortisol on smolt behavior, [Iwata \(1995\)](#) concluded that thyroid hormones had clear effects on the salinity preference of smolts but that effects on downstream migration were less certain. In experiments on chum salmon fry, T_4 treatment induced higher levels of circulating T_4 but had no effect on downstream movement ([Ojima and Iwata, 2007b](#)). Intracerebroventricular injections of gonadotropin-releasing hormone (GnRH) and CRH in juvenile coho salmon

resulted in both downstream movement and increased plasma T_4 levels, leading the authors to suggest that increases in thyroid hormones during migration are an *outcome* of other neuroendocrine hormones rather than a driving factor for migration (Ojima and Iwata, 2010).

Long-term dietary or injection treatments with thyroid hormones have only a limited impact on salinity tolerance of salmonids (McCormick, 2001). This contrasts with cortisol and GH, which as noted above can individually increase salt secretory capacity and together have an additive or synergistic effect. It should be noted that the repressor nature of thyroid hormone receptors may make it difficult to utilize exogenous hormone treatments, and negative effects should be viewed with some skepticism. However, the fact that some aspects of smolt development (e.g. morphology and metabolism) can be altered by exogenous thyroid hormone treatment indicates that the development of salinity tolerance is less likely to be under direct control of thyroid hormones. Rather, thyroid hormones are more likely to have a permissive role or to work indirectly through the GH–IGF-I and cortisol axes (Takei and McCormick, 2013, Chapter 3, this volume). For instance, exogenous T_3 treatment increases gill cortisol receptors and synergizes with GH to increase them even further (Shrimpton and McCormick, 1998a).

The hypothalamic control of thyroid hormones during smolt development has not been examined. Larsen et al. (1998) have provided convincing evidence that CRF, and not thyroid-releasing hormone or GHRH, is the most potent stimulator of thyroid-stimulating hormone (TSH) release in coho salmon parr. Thus, the increased CRF neurogenesis in Atlantic salmon smolts noted above may play a role in thyroid hormone regulation. In coho salmon smolts, pituitary TSH mRNA levels decrease in spring, whereas pituitary and plasma levels of TSH do not change and plasma thyroid hormones increase (Larsen et al., 2011). The capacity of mammalian thyroid stimulating hormone to increase plasma T_4 changes during smolt development, peaking at or near the time of downstream migration (Specker and Schreck, 1984). Thus, the seasonal changes in plasma T_4 may owe more to changes in sensitivity to TSH than to changes in TSH itself. Metabolic clearance rate of thyroid hormones is greatest early in smolt development when plasma T_4 levels are low, but subsequently decreases (Ojima and Iwata, 2007a). These authors suggest that the thyroid surge seen in some species near the peak of smolt development is due to lower tissue utilization of T_4 and T_3 , consistent with observations on thyroid kinetics during smolt development (Specker et al., 1984). The importance of intracellular levels of thyroid hormones (and thus deiodinases) during smolt development has been emphasized by Specker et al. (1992), although a clear picture of their regulation and relation to thyroid hormone action has yet to emerge.

7. DEVELOPMENTAL AND ENVIRONMENTAL REGULATION

7.1. Development and Heterochrony

Rounsefell (1958) was the first to quantify the relative reliance on FW and SW (the “scale of anadromy”) among salmonid species and genera, with a general increase in life history spent in SW among the genus *Oncorhynchus* (Fig. 5.8A). The increased proportion of time spent in SW is primarily driven by an earlier migration from FW to SW, which not surprisingly is also accompanied by earlier development of SW tolerance. This has been pointed out to be a heterochrony, in which the timing of developmental events (smolting and downstream migration) has been shifted earlier in more recently evolved species (McCormick, 1994) (Fig. 5.8B). Thus, species such as Atlantic salmon and steelhead trout spend one to several years in FW before undergoing a size-dependent transformation at 12–15 cm, whereas pink and chum salmon may spend as little as a few weeks after hatching in FW and begin their seaward migration at a much smaller size of 3–5 cm. Based on a consensus phylogeny in which *Salvelinus* is basal and *Salmo* and *Oncorhynchus* are sister genera, this suggests an evolutionary pattern in which salinity tolerance has been shifted to earlier developmental stages, especially within the most recently derived species of *Oncorhynchus* (Fig. 5.8). In heterochronic terms, this is a peramorphosis caused by predisplacement, the earlier onset of a developmental event.

In addition to the shift in timing, a change in the environmental factors that regulate smolt development has occurred (McCormick, 1994; Gallagher et al., 2013). In facultatively anadromous species such as brook trout and lake trout, smolt development is poorly developed or absent, and salinity itself is the primary inducer of mechanisms for salt tolerance. In smolting salmonids that spend at least a year in FW (Atlantic salmon, steelhead trout, coho salmon), a minimum size must be achieved, after which the animals alter their response to photoperiod. Thus, in Atlantic salmon a threshold size of 12–13 cm must be achieved by late January when daylength begins to increase (McCormick et al., 2007). In coho and Chinook salmon a growth threshold may also be involved in the initial “decision” to become smolts (Dickhoff et al., 1997; Beckman et al., 2007). If fish have not achieved this minimum size they will wait another year (or more) before transforming to smolts. In Atlantic salmon the attainment of smolt size as 1-year olds (1+) has been related to a bimodal growth pattern that develops several months before, in their first autumn (Kristinsson et al., 1985). How fish determine their size and/or growth rate at the appropriate time of year and translate this to increased responsiveness to daylength is an important area that has not received substantial attention. It has been suggested that proxies for size

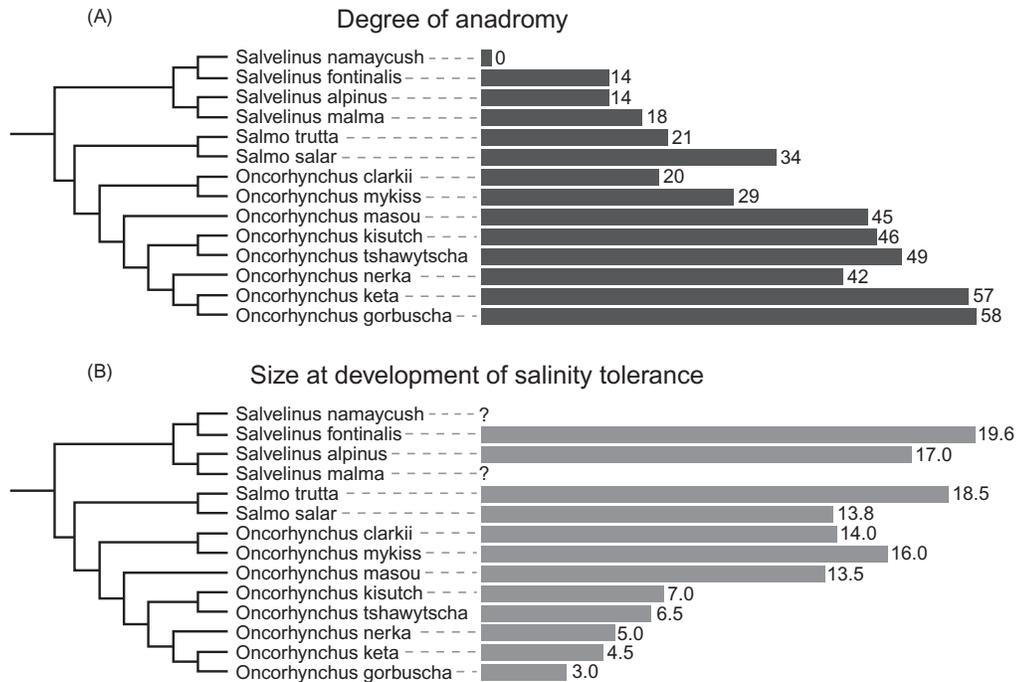


Fig. 5.8. Phylogeny of the subfamily Salmoninae mapped with the degree of anadromy (A) and the size at which salinity tolerance develops (B). The figures demonstrate that there is increased anadromy in more recently evolved salmonines, and that there has been a heterochrony in the size (and age) of smolt development with a clear trend towards smaller and earlier development of salinity tolerance and accompanying seaward migration. Phylogeny is a consensus phylogeny incorporating genetic, morphological, and life history data from 38 separate analyses in 34 published studies (Wilson and Williams, 2010). Data for the degree of anadromy are from Rounsefell (1958), with the exception of masu salmon, which was based on life history characteristics presented by Kato (1991). The degree of anadromy is based on six criteria, each with a maximum 10-point score: (1) extent of migration in the sea; (2) duration of stay in the sea; (3) state of maturity attained at sea; (4) spawning habits and habitat; (5) mortality after spawning; and (6) occurrence of freshwater (FW) forms. Size at development of salinity tolerance was taken from literature values in which fish were directly transferred from FW to at least 30 ppt seawater and survival was monitored for at least 14 days and was greater than 75% (with the exception of *S. fontinalis* in which a gradual acclimation was employed, and *O. nerka* in which survival was only monitored for 1 day); *S. fontinalis* (McCormick and Naiman, 1984), *S. alpinus* (Arnesen et al., 1992), *S. salar* (Johnston and Saunders, 1981), *S. trutta* (Parry, 1960), *O. mykiss* (Conte and Wagner, 1965), *O. clarkii* (Yeoh et al., 1991), *O. masou* (Ban et al., 1988), *O. tshawytscha* (Wagner et al., 1969), *O. kisutch* (Conte et al., 1966), *O. nerka* (Heifetz et al., 1989), *O. keta* (Black, 1951), *O. gorbuscha* (Gallagher et al., 2013). Phylogeny was created with software developed by Letunic and Bork (2011).

and growth such as energy reserves or fat content signaled through metabolic or neuroendocrine pathways may be involved (McCormick, 1994), but these have yet to be established.

In pink and chum salmon the early development of salinity tolerance, occurring soon after hatching, appears to be largely independent of

environmental factors and to be hardwired to occur (McCormick, 1994; Gallagher et al., 2013). In addition to species difference, there appears to be diversity in the size and time of year at which smolt development and migration occur within the species, especially in Chinook salmon, and diversity can occur within and between populations (Taylor, 1990). Thus, for the following sections on environmental factors that regulate physiological and behavioral aspects of smolt development it is important to point out that the critical environmental cues are likely to differ among species and among populations within species. Extensive reviews on environmental factors controlling smolt development have been published by Hoar (1988) and Björnsson et al. (2011), the latter focusing on those factors most relevant to fish in the wild.

7.2. Photoperiod

For those smolting salmonids that migrate at least a year after hatching, photoperiod has been shown to be the most important environmental determinant for the timing of virtually all physiological and behavioral aspects of smolt development (Hoar, 1988). In Atlantic salmon, advanced increases in daylength or out-of-phase photoperiod can advance smolting by many months (Duston and Saunders, 1995). Although there is some evidence for an endogenous rhythm of smolting (Eriksson and Lundqvist, 1982), increased daylength is probably necessary for normal smolting to occur, and is certainly required for its normal timing. The capacity of photoperiod to affect smolting may be restricted at low temperatures (McCormick et al., 2000), indicating that temperature may be a controlling factor in smolting (Fry, 1971), at least in some species.

The actions of photoperiod on the timing of smolt development are cued by its impact on the neuroendocrine axis (Björnsson et al., 2011). Circulating levels of GH, cortisol, and thyroid hormones have all been shown to be responsive to changes in photoperiod. GH seems to be especially responsive, increasing within several days of an advanced photoperiod, and potentially causing a cascade of other endocrine events (McCormick et al., 1995). Atlantic salmon parr too small to undergo smolting do not change plasma GH or cortisol substantially in spring, nor do they respond to an advanced photoperiod; in contrast, fish that have reached the critical size for smolting in this species increased plasma GH and cortisol in the spring, the timing of which can be advanced by increased daylength (McCormick et al., 2007). Thus, developmental difference in the capacity to respond to photoperiod is a key component of smolting in this species. This developmental difference may be due in part to changes in retinal and pineal innervation of the hypothalamus. Atlantic salmon smolts

develop robust retinal innervation of the preoptic nucleus, an area known to regulate pituitary function (Ebbesson et al., 2007). Furthermore, this innervation is blocked by exposure to continuous light, which also inhibits normal smolt development.

The mechanism by which photoperiodic information is translated to a neuroendocrine response in teleosts has not been established. Melatonin secretion by the pineal of salmonids can be directly entrained by photoperiod, but the relationship of melatonin rhythm to photoperiodism in fish is unclear (Falcon et al., 2007). The major clock genes found in other vertebrates (Clock, Bmal1, Per2, and Cry2) are expressed in the brains of juvenile Atlantic salmon and can be altered by changes in daylength (Davie et al., 2009). Diel rhythms of plasma melatonin and cortisol and expression patterns of brain and pineal Clock, Cry2, and Per1 were present in presmolt Atlantic salmon exposed to short days but were abolished after exposure to increased daylength (Huang et al., 2010). The presence of latitudinal variation in the OtsClock1b gene in four populations of Pacific salmon is hypothesized to play a role in the timing of spawning, although it is possible that it also plays a role in other seasonal life history events such as smolting and downstream migration. Further work is clearly needed to determine the mechanistic link among changes in daylength, neuroendocrine activation, and physiological and behavioral responses.

As noted above, photoperiod is the seasonal “zeitgeber” that triggers a complex series of developmental events that precede and prepare salmon for migration. Although photoperiod influences the timing of migratory behavior of salmon smolts (Hoar, 1976), it is not the only environmental mediator. Several authors have proposed that photoperiod may act to increase activity (migratory restlessness or Zugunruhe) and make fish sensitive to other environmental cues (releasing factors) that initiate migration (McCormick et al., 1998). Photoperiod will therefore determine the range of days or weeks during which migration may occur, but other factors such as water temperature, flow, and turbidity may act as releasing factors within that range.

7.3. Temperature

Studies using differing levels of constant temperature during smolt rearing have found that increased temperature results in earlier smolt development in Atlantic salmon and steelhead trout (although at temperatures of 15°C and higher, smolt development of steelhead is inhibited) (Adams et al., 1973; Johnston and Saunders, 1981; Solbakken et al., 1994). Exposure of Atlantic salmon smolts to several rates of temperature increase in spring confirms this effect of temperature and

indicates a strong relationship between the cumulative temperature experience (degree days) and smolt development (Sigholt et al., 1998), indicating that temperature is acting to control the rate of development. Effects of temperature on smolt development may not be universal, as Larsen et al. (2001) found no difference in the timing or magnitude of changes in gill NKA activity of coho salmon smolts reared at 2.5 and 10°C in winter and spring. In reviewing the influence of temperature on smolt development, McCormick et al. (1997) concluded that although increased temperature was not necessary for the completion of smolting, it acted with photoperiod to advance smolting and could act as a directive factor in the absence of photoperiod cues. However, more recent studies indicate that temperature acts primarily on the rate of development and has only a limited ability to act as a directive factor. Under normal daylength conditions, an advanced temperature regimen resulted in more rapid increases in gill NKA activity, but the date at which increases began and peak levels occurred was the same for the two temperature regimens (McCormick et al., 2002). The same study found that smolting remained incomplete under a regimen of short days and an advanced temperature regimen, indicating that temperature increase alone cannot act to advance smolting in the absence of photoperiod cues.

Developmental events and circannual cues, working through the neuroendocrine axis, set the stage for both physiological development and migratory readiness. Environmental factors can then act as “releasers” of migratory behavior (Baggerman, 1960), as outlined in Fig. 5.7. Which hormones are most important for determining migratory readiness has not been examined. As noted above, temperature has a role in determining the timing of smolt development (and thus migratory readiness), but is also likely to have a role as a releasing factor. However, determining the independent effects of temperature on these two processes may prove to be difficult. A large number of field studies have found a correlation between migration timing and temperature, and it seems clear that water temperature is an important factor, perhaps the most important factor, initiating downstream migrations in salmon (Jonsson et al., 1991; McCormick et al., 1998). Several authors have reported that downstream migration of smolts of Atlantic salmon and brown trout occurs at about 10°C or slightly above, and similar aspects of threshold temperatures have been proposed for smolt migration in other species (Jonsson et al., 1991). There are several examples, however, of populations of Pacific salmon in northern latitudes beginning or even completing migration before these thresholds are reached (J. M. Shrimpton, personal communication). Thus, there is some reason to question whether a temperature threshold is a major driver of smolt migration. Zydlewski et al. (2005) found that the effect of an advanced

temperature regimen on both the onset and termination of migratory behavior in captive Atlantic salmon smolts could best be explained by accumulated thermal units (ATUs, also known as cumulative degree days) rather than a threshold temperature. Similarly, the effect of temperature manipulation on migration of captive Chinook salmon smolts was related to ATUs. Analysis of a 12 year dataset of wild Chinook salmon smolt migration timing found that ATUs had a greater explanatory power than a threshold temperature (Sykes et al., 2009). In addition to cumulative degree days there may be other aspects of temperature such as the magnitude of diel changes or population-specific thresholds that play a role in migration.

7.4. Flow and Turbidity

High water flow in rivers may stimulate downstream movement in a large number of fish species (Jonsson et al., 1991), and the downstream migration of smolts has been linked to increased water flow (see references in McCormick et al., 1998). As pointed out above, it is often difficult to separate the impact of flow on the initiation and the speed of migration in field studies. The migration of Atlantic salmon smolts in the River Orkla, Norway, was coincident with the first spring peak in water discharge when the water temperature was above 3°C (Hesthagen and Garnas, 1986). It has been suggested that smolt migration is a result of a passive displacement by high water flow (Thorpe, 1984), although this hypothesis has not received substantial empirical support (Bourgeois and O'Connell 1988). It has been clearly demonstrated that smolts migrate more quickly at high water velocities than at low flow (Youngson et al., 1989). Using an artificial tank system to examine downstream migration, Sykes and Shrimpton (2010) found that temperature rather than flow most strongly influenced the initiation of migration. However, the presence of a strong, directional current resulted in a period of more defined movement, indicating an influence of current once migration is underway. Atlantic salmon smolts actively move out into the main current of the river, apparently to avoid being caught in backwaters and sloughs (Hansen and Jonsson, 1985). Smolts migrating through lakes move faster than the downstream current velocity (Bourgeois and O'Connell 1988), further indicating that the seaward migration is not purely passive.

There is strong evidence that the survival of hatchery-reared smolts improves significantly when they are released at high water discharge within the normal period of migration (Hosmer et al., 1979; Hvidsten and Hansen, 1988). Wild coho salmon survival (as measured by adult return rates) is highest in years with high flow rates during spring emigration (Lawson et al., 2004), further suggesting an important link between flow and survival.

Several factors may contribute to this greater survival associated with high flow, including higher migration speeds and reduced visibility due to turbidity, both of which can reduce predation (Gregory and Levings, 1998). Greater migration speeds may also minimize the chance that fish may enter the ocean after they have reached the peak of SW adaptability.

7.5. Physiological and Ecological Smolt Windows

Salinity tolerance, migratory behavior, and several other aspects of development that occur during smolting are lost if fish remain in FW (Fig. 5.2A,B). This process has been called “desmoltification”, although this term should be used with some caution, as it implies that all aspects of smolting are reversed, which is not the case. Nonetheless, many of the reversible aspects of smolting such as salinity tolerance are critical for marine survival, and indicate that there is a limited period during which the fish are at peak preparedness for SW entry, known as the physiological smolt window. The boundaries of the physiological smolt window will be determined by the environmental factors that control the stimulation and loss of smolt characters. As noted above, photoperiod and temperature are the primary drivers of smolt development in coho, steelhead, and Atlantic salmon, whereas photoperiod may be less important in pink and chum salmon that develop salinity tolerance soon after hatching. In coho, steelhead, and Atlantic salmon, it appears that the loss of salinity tolerance and other aspects of smolt development are driven primarily by temperature and time (Zaugg and McLain, 1976; Duston et al., 1991). This relationship has been investigated in detail in Atlantic salmon, where there is a dome-shaped relationship between cumulative degree days and the development and loss of salinity tolerance and/or gill NKA activity (McCormick et al., 1997). Thus, after peak smolt development is reached, fish that experience an additional 500 ATU will have reverted back to parr levels of salinity tolerance. It appears that the regulation of smolt behavior is similar to that relating to salinity tolerance, in that higher temperatures lead to an earlier cessation of downstream migratory behavior (Wagner, 1974; Zydlewski et al., 2005).

Adult return rates of hatchery-reared salmon smolts, perhaps the most important measure of SW performance, are strongly dependent on the timing of release. Hatchery-reared Atlantic salmon released in late May during the normal migratory period of wild fish have higher adult return rates than fish released in April and June (Staurnes et al., 1993; Lundqvist et al., 1994). Similarly, Bilton et al. (1982) found substantially higher return rates in hatchery-reared coho salmon released in April and May than in June and July. In studies where it is measured, the peak of physiological

smolt development corresponds with the timing of release that yields the highest adult return rates (Virtanen et al., 1991). This correspondence is likely to be dependent on both the physiological smolt window and coincidence of release and subsequent migration with optimum environmental conditions for smolt survival (the “ecological smolt window”) (McCormick et al., 1998). It seems likely that natural selection will have shaped the physiological smolt window to coincide with the ecological smolt window. Stock-specific differences in the timing of downstream migration (Orciari and Leonard, 1996; McGinnity et al., 2007) or increases in salinity tolerance (Handeland et al., 2004) are likely examples of selection acting on the timing of smolt development.

In addition to the timing of release, FW rearing conditions in hatcheries will affect the overall SW performance of smolts. Beckman et al. (1999) found that the same strain of spring Chinook salmon reared at three different hatcheries had different smolt-to-adult return rates, and that these were most strongly correlated with spring growth, plasma IGF-I levels, and gill NKA activity. Fish density and raceway flows experienced by juveniles in FW affect the adult return rates of spring Chinook salmon (Banks, 1994). Several studies have found a positive correlation between size of released smolts, and smolt survival and adult return rates (Miyakoshi et al., 2001; Connor et al., 2004). The effect of size appears to be more pronounced in years with poor overall marine survival (Saloniemi et al., 2004). The effects of rearing conditions are generally smaller than the effects of time of release, but clearly indicate that growth conditions in FW affect smolt survival. While these studies have focused on hatcheries, they underscore the principle that variations in environmental conditions in streams and rivers impact smolt development and SW performance, and it would be of interest to determine whether variations in winter and spring growth conditions affect survival in wild fish.

How the physiological smolt window affects marine survival is likely to be more complex than smolts simply suffering acute osmoregulatory failure and dying following movement into high-salinity waters. It is more likely that there will be a more subtle compromise in their ability to migrate, forage, and avoid predators, and an increase in time spent in estuaries where predation pressures are high. Minor compromises of physiological and/or behavioral preparation which have minimal effects in the laboratory may have dire consequences in the wild. Mortality rates of up to 34% due to predation during the first 2 days of SW entry have been reported in brown trout smolts (Dieperink et al., 2002). Atlantic salmon smolts directly transferred to SW suffered greater experimental predation in SW than smolts acclimated to SW for 30 days (Jarvi, 1990). Similarly, the greatest predation rates on smolts occurred during the first days of exposure to SW

when plasma ion perturbations were at their highest levels (Handeland et al., 1996). Chinook salmon smolts subjected to a handling stress spent more time in the upper, FW layers of a simulated estuary than did unstressed fish (Price and Schreck, 2003), which the authors suggest would expose them to greater avian predation. Furthermore, lower levels of gill NKA activity are associated with greater levels of avian predation in estuaries during downstream migration of Chinook salmon (Schreck et al., 2006). Osmotic and other stressors may also result in increased susceptibility to diseases that reduce estuarine and marine survival (Loge et al., 2005). Adult return rates of coho salmon subjected to transportation stress just before release were reduced compared to those of fish released directly from the hatchery or those allowed 6 weeks to recover from transportation stress (Schreck et al., 1989). A variety of contaminants have been shown to affect smolt development, some of which specifically act on salinity tolerance and the capacity for marine survival (McCormick et al., 2009a). All of these studies indicate that factors compromising the physiological and behavioral capacity of smolts to make the transition from FW to SW are likely to result in an overall decrease in marine survival, even if the ultimate source of mortality is not directly due to osmoregulatory failure.

8. CONCLUSIONS AND PERSPECTIVES

The migration of smolts takes them from headwater streams, through large rivers, into estuaries, and out into the expansive ocean. Smolts can therefore be affected by natural and anthropogenic changes that affect any one of these aquatic environments. Sedimentation and pollution can degrade upland aquatic habitats. Dams and turbines can be directly lethal, but also alter migration patterns and affect the thermal environment experienced by smolts. Invasion of estuarine habitats by a new competitor, predator, or disease agent can increase mortality of smolts at a stage when mortality is already high. Natural variations in ocean conditions are also known to alter smolt survival and long-term alterations induced by climate change may cause a shift to less favorable conditions.

To understand the impacts of environmental change on salmon smolts, it will be critical to understand both their basic biology and their susceptibility to threats. There are large variations in the responses of individual species to environmental change. As this chapter has shown, smolts undergo a variety of preparatory developmental changes that allow them to migrate and live in dramatically different habitats. The control of migration timing appears to be critical for smolt survival (Thorstad et al., 2012), and knowledge of how

climate change will affect the ecological and physiological smolt windows will be critical to predicting the future of salmon populations (McCormick et al., 1998). The incredible plasticity of smolts has some tradeoffs, and in some cases appears to make them more susceptible to external stressors. For instance, smolts are more sensitive than other life stages to the impacts of acidification (Rosseland et al., 2001). Exposure to contaminants in streams and rivers can affect not only FW survival, but the ability to make the transition to the marine environment (McCormick et al., 2009a). Climate change is likely to interact with other habitat alterations to compound negative effects on smolt survival and salmon persistence. Continued examination of the biology of smolts and their response to environmental changes will be critical to understanding their habitat requirements and conservation needs for ensuring salmon survival.

ACKNOWLEDGMENTS

I thank all of the members of my lab and my collaborators over the years for their willingness to share ideas, some of which have probably appeared here unknowingly without proper attribution. Thanks to Eric Schultz, Arne Christensen, Amy Regish, Michael O'Dea, and Tara Duffy for their help in composing several of the figures. I dedicate this chapter to Howard Bern, mentor, friend, and forever a source of inspiration.

REFERENCES

- Adams, B. L., Zaugg, W. S. and McLain, L. R. (1973). Temperature effect on parr-smolt transformation in steelhead trout (*Salmo gairdneri*) as measured by gill sodium-potassium stimulated ATPase. *Comp. Biochem. Physiol. [A]* 44, 1333–1339.
- Agustsson, T., Sundell, K., Sakamoto, T., Johansson, V., Ando, M. and Bjornsson, BT. (2001). Growth hormone endocrinology of Atlantic salmon (*Salmo salar*): pituitary gene expression, hormone storage, secretion and plasma levels during parr-smolt transformation. *J. Endocr.* 170, 227–234.
- Arnesen, A. M., Halvorsen, M. and Nilssen, K. J. (1992). Development of hypoosmoregulatory capacity in Arctic char (*Salvelinus alpinus*) reared under either continuous light or natural photoperiod. *Can. J. Fish. Aquat. Sci.* 49, 229–237.
- Baggerman, B. (1960). Factors in the diadromous migrations of fish. *Zool. Soc. Symp., London I* 33–60.
- Ban, M., Kanno, H., Izumi, T. and Yamauchi, K. (1988). Body size and seawater tolerance in the hatchery-reared underyearling masu salmon (*Oncorhynchus masou*). *Tohoku Reg. Fish. Res. Lab. Bull.* 50, 117–123.
- Bandoh, H., Kida, I. and Ueda, H. (2011). Olfactory responses to natal stream water in sockeye salmon by BOLD fMRI. *PLOS ONE* 6, e16051.
- Banks, J. L. (1994). Raceway density and water flow as factors affecting spring chinook salmon (*Oncorhynchus tshawytscha*) during rearing and after release. *Aquaculture* 119, 201–217.

- Beckman, B. R. and Dickhoff, W. W. (1998). Plasticity of smolting in spring chinook salmon: relation to growth and insulin-like growth factor-I. *J. Fish Biol.* 53, 808–826.
- Beckman, B. R., Dickhoff, W. W., Zaugg, W. S., Sharpe, C., Hirtzel, S., Schrock, R., Larsen, D. A., Ewing, R. D., Palmisano, A., Schreck, C. B. and Mahnken, C. W. (1999). Growth, smoltification, and smolt-to-adult return of spring chinook salmon from hatcheries on the Deschutes River, Oregon [Review]. *Trans. Am. Fish. Soc.* 128, 1125–1150.
- Beckman, B. R., Gadberry, B., Parkins, P., Cooper, K. A. and Arkush, K. D. (2007). State-dependent life history plasticity in Sacramento River winter-run chinook salmon (*Oncorhynchus tshawytscha*): interactions among photoperiod and growth modulate smolting and early male maturation. *Can. J. Fish. Aquat. Sci.* 64, 256–271.
- Beeman, J. W., Rondorf, D. W. and Tilson, M. E. (1994). Assessing smoltification of juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) using changes in body morphology. *Can. J. Fish. Aquat. Sci.* 51, 836–844.
- Bern, H. A. (1978). Endocrinological studies on normal and abnormal salmon smoltification. In *Comparative Endocrinology* (eds. P. J. Gaillard and H. H. Boer), pp. 77–100. Amsterdam: Elsevier/North Holland Biomedical Press.
- Bertmar, G. (1983). Monthly development of Baltic salmon olfactory organs before and during the imprinting period, and comparisons with precocious males of the same stock. *Aquillo Ser. Zool.* 22, 37–44.
- Bilton, H. T., Alderdice, D. F. and Schnute, J. T. (1982). Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. *Can. J. Fish. Aquat. Sci.* 39, 426–447.
- Björnsson, B. Th., Stefansson, S. O. and McCormick, S. D. (2011). Environmental endocrinology of salmon smoltification. *Gen. Comp. Endocrinol.* 170, 290–298.
- Black, V. S. (1951). Changes in body chloride, density and water content of chum (*Oncorhynchus keta*) and coho (*O. kisutch*) salmon fry when transferred from fresh water to sea water. *J. Fish. Res. Board Can.* 8, 164–177.
- Boeuf, G. (1993). Salmonid smolting: a pre-adaptation to the oceanic environment. In *Fish ecophysiology* (eds. J. C. Rankin and F. B. Jensen), pp. 105–135. London: Chapman and Hall.
- Bourgeois, C. E. and O'Connell, M. F. (1988). Observations on the seaward migration of Atlantic salmon (*Salmo salar* L.) smolts through a large lake as determined by radiotelemetry and Carlin tagging studies. *Can. J. Zool.* 66, 685–691.
- Brannon, E. L. and Quinn, T. P. (1990). Field test of pheromone hypothesis for homing of pacific salmon. *J. Chem. Ecol.* 16, 603–610.
- Brauner, C. J., Shrimpton, J. M. and Randall, D. J. (1992). Effect of short-duration seawater exposure on plasma ion concentrations and swimming performance in coho salmon (*Oncorhynchus kisutch*) parr. *Can. J. Fish. Aquat. Sci.* 49, 2399–2405.
- Carey, J. B. and McCormick, S. D. (1998). Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. *Aquaculture* 168, 237–253.
- Clarke, W. C., Saunders, R. L. and McCormick, S. D. (1996). Smolt Production. In *Principles of Salmonid Culture*, vol. 29 (eds. W. Pennel and B. A. Barton), pp. 517–567. Amsterdam: Elsevier.
- Clements, S. and Schreck, C. B. (2004). Central administration of corticotropin-releasing hormone alters downstream movement in an artificial stream in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Gen. Comp. Endocrinol.* 137, 1–8.
- Collie, N. L. and Bern, H. A. (1982). Changes in intestinal fluid transport associated with smoltification and seawater adaptation in coho salmon, *Oncorhynchus kisutch* (Walbaum). *J. Fish Biol.* 21, 337–348.
- Connor, W. P., Smith, S. G., Andersen, T., Bradbury, S. M., Burum, D. C., Hockersmith, E. E., Schuck, M. L., Mendel, G. W. and Bugert, R. M. (2004). Postrelease performance of

- hatchery yearling and subyearling fall chinook salmon released into the Snake River. *N. Am. J. Fish. Manag.* 24, 545–560.
- Conte, F. P. and Wagner, H. H. (1965). Development of osmotic and ionic regulation in juvenile steelhead trout *Salmo gairdneri*. *Comp. Biochem. Physiol.* 14, 603–620.
- Conte, F. P., Wagner, H. H., Fessler, J. and Gnose, C. (1966). Development of osmotic and ionic regulation in juvenile coho salmon (*Oncorhynchus kisutch*). *Comp. Biochem. Physiol.* 18, 1–15.
- Dauder, S., Young, G., Hass, L. and Bern, H. A. (1990). Prolactin receptors in liver, kidney, and gill of the tilapia (*Oreochromis mossambicus*): characterization and effect of salinity on specific binding of iodinated ovine prolactin. *Gen. Comp. Endocrinol.* 77, 368–377.
- Davie, A., Minghetti, M. and Migaud, H. (2009). Seasonal variations in clock-gene expression in Atlantic salmon (*Salmo salar*). *Chronobiol. Int.* 26, 379–395.
- Dickhoff, W. W., Beckman, B. R., Larsen, D. A., Duan, C. and Moriyama, S. (1997). The role of growth in endocrine regulation of salmon smoltification. *Fish Physiol. Biochem.* 17, 231–236.
- Dickhoff, W. W., Folmar, L. C. and Gorbman, A. (1978). Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 36, 229–232.
- Dickhoff, W. W. and Sullivan, C. V. (1987). Involvement of the thyroid gland in smoltification, with special reference to metabolic and developmental processes. *Am. Fish. Soc. Symp.* 1, 197–210.
- Dieperink, C., Bak, B. D., Pedersen, L. F., Pedersen, M. I. and Pedersen, S. (2002). Predation on Atlantic salmon and sea trout during their first days as postsmolts. *J. Fish Biol.* 61, 848–852.
- Dittman, A. H. and Quinn, T. P. (1996). Homing in Pacific salmon: Mechanisms and ecological basis. *J. Exp. Biol.* 199, 83–91.
- Dittman, A. H., Quinn, T. P. and Nevitt, G. A. (1996). Timing of imprinting to natural and artificial odors by coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* 53, 434–442.
- Dittman, A. H., Quinn, T. P., Nevitt, G. A., Hacker, B. and Storm, D. R. (1997). Sensitization of olfactory guanylyl cyclase to a specific imprinted odorant in coho salmon. *Neuron* 19, 381–389.
- Duan, C. M., Plisetskaya, E. M. and Dickhoff, W. W. (1995). Expression of insulin-like growth factor I in normally and abnormally developing coho salmon (*Oncorhynchus kisutch*). *Endocrinol.* 136, 446–452.
- Dukes, J. P., Deaville, R., Bruford, M. W., Youngson, A. F. and Jordan, W. C. (2004). Odorant receptor gene expression changes during the parr-smolt transformation in Atlantic salmon. *Molec. Ecol.* 13, 2851–2857.
- Duston, J. and Saunders, R. L. (1995). Advancing smolting to autumn in age 0+ Atlantic salmon by photoperiod, and long-term performance in sea water. *Aquaculture* 135, 295–309.
- Duston, J., Saunders, R. L. and Knox, D. E. (1991). Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 48, 164–169.
- Ebbesson, L. O. E., Ebbesson, S. O. E., Nilsen, T. O., Stefansson, S. O. and Holmqvist, B. (2007). Exposure to continuous light disrupts retinal innervation of the preoptic nucleus during parr-smolt transformation in Atlantic salmon. *Aquaculture* 273, 345–349.
- Ebbesson, L. O. E., Nilsen, T. O., Helvik, J. V., Tronci, V. and Stefansson, S. O. (2011). Corticotropin-releasing factor neurogenesis during midlife development in salmon: genetic, environmental and thyroid hormone regulation. *J. Neuroendocrinol.* 23, 733–741.
- Eddy, F. B. and Talbot, C. (1985). Urine production in smolting Atlantic salmon, *Salmo salar* L. *Aquaculture* 45, 67–72.

- Edwards, S. L. and Marshall, W. S. (2013). Principles and patterns of osmoregulation and euryhalinity in fishes. In *Fish Physiology*, Vol. 32, *Euryhaline Fishes* (eds. S. D. McCormick, C. J. Brauner and A. P. Farrell). Amsterdam: Academic Press, pp. 1–44.
- Eriksson, L.-O. and Lundqvist, H. (1982). Circannual rhythms and photoperiod regulation of growth and smolting in Baltic salmon (*Salmo salar* L.). *Aquaculture* 28, 113–121.
- Evans, D. H., Piermarini, P. M. and Choe, K. P. (2005). The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiol. Rev.* 85, 97–177.
- Falcon, J., Besseau, L., Sauzet, S. and Boeuf, G. (2007). Melatonin effects on the hypothalamo-pituitary axis in fish. *Trends Endocrinol. Metab.* 18, 81–88.
- Fontaine, M. and Hatey, J. (1954). Sur la teneur en 17-hydroxycorticosteroides du plasma de saumon (*Salmo salar* L.). *Compt. Rend. Acad. Sci.* 239, 319–321.
- Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In *Fish Physiology*, Volume VI, *Environmental Relations and Behavior* (eds. W. S. Hoar and D. J. Randall), pp. 1–98. New York: Academic Press.
- Gallagher, Z., Bystriansky, J.S., Farrell, A.P. and Brauner, C.J. (2013). A novel pattern of smoltification in the most anadromous salmonid: Pink salmon (*Oncorhynchus gorbuscha*). *Can. J. Fish. Aquat. Sci.* (in press).
- Gibson, R. J. (1983). Water velocity as a factor in the change from aggressive to schooling behaviour and subsequent migration of Atlantic salmon smolt (*Salmo salar*). *Le Naturaliste Canadien* 110, 143–148.
- Gorbman, A., Dickhoff, W. W., Mighell, J. L., Prentice, E. F. and Waknitz, F. W. (1982). Morphological indices of developmental progress in the parr-smolt coho salmon, *Oncorhynchus kisutch*. *Aquaculture* 28, 1–19.
- Grau, E. G., Dickhoff, W. W., Nishioka, R. S., Bern, H. A. and Folmar, L. C. (1981). Lunar phasing of the thyroxine surge preparatory to seaward migration of salmonid fish. *Science* 211, 607–609.
- Gregory, R. S. and Levings, C. D. (1998). Turbidity reduces predation on migrating juvenile Pacific salmon. *Trans. Am. Fish. Soc.* 127, 275–285.
- Grossell, M. (2011). In *The role of the gastrointestinal tract in salt and water balance* (eds. M. Grossell, A. P. Farrell and C. J. Brauner), pp. 135–164. London: Elsevier.
- Handeland, S. O., Jarvi, T., Ferno, A. and Stefansson, S. O. (1996). Osmotic stress, antipredator behaviour, and mortality of Atlantic salmon (*Salmo salar*) smolts. *Can. J. Fish. Aquat. Sci.* 53, 2673–2680.
- Handeland, S. O., Wilkinson, E., Sveinsbo, B., McCormick, S. D. and Stefansson, S. O. (2004). Temperature influence on the development and loss of seawater tolerance in two fast-growing strains of Atlantic salmon. *Aquaculture* 233, 513–529.
- Hansen, L. P. and Jonsson, B. (1985). Downstream migration of hatchery-reared smolts of Atlantic salmon (*Salmo salar* L.) in the River Imsa. *Aquaculture* 45, 237–248.
- Hansen, L. P. and Quinn, T. R. (1998). The marine phase of the Atlantic salmon (*Salmo salar*) life cycle, with comparisons to Pacific salmon. *Can. J. Fish. Aquat. Sci.* 55, 104–118.
- Hara, T. J. (1994). Olfaction and gustation in fish: An overview. *Acta Physiol. Scand.* 152, 207–217.
- Hasler, A. D. and Scholz, A. T. (1983). *Olfactory Imprinting and Homing in Salmon*. New York: Springer-Verlag.
- Heifetz, J., Johnson, S. W., Koski, K. V. and Murphy, M. L. (1989). Migration timing, size, and salinity tolerance of sea-type sockeye salmon (*Oncorhynchus nerka*) in an Alaska estuary. *Can. J. Fish. Aquat. Sci.* 46, 633–637.

- Hesthagen, T. and Garnas, E. (1986). Migration of Atlantic salmon smolts in River Orkla, central Norway in relation to management of a hydroelectric station. *N. Am. J. Fish. Manag.* 6, 376–382.
- Hoar, W. S. (1939). The thyroid gland of the Atlantic salmon. *J. Morphol.* 65, 257–295.
- Hoar, W. S. (1976). Smolt transformation: evolution, behavior, and physiology. *J. Fish. Res. Board Can.* 33, 1234–1252.
- Hoar, W. S. (1988). The physiology of smolting salmonids. In *Fish Physiology*, Vol. XIB (eds. W. S. Hoar and D. Randall), pp. 275–343. New York: Academic Press.
- Hoffnagle, T. L. and Fivizzani, A. J. (1990). Stimulation of plasma thyroxine levels by novel water chemistry during smoltification in chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 47, 1513–1517.
- Hogasen, H. R. and Prunet, P. (1997). Plasma levels of thyroxine, prolactin, and cortisol in migrating and resident wild arctic char, *Salvelinus alpinus*. *Can. J. Fish. Aquat. Sci.* 54, 2947–2954.
- Hosmer, M. J., Stanley, J. G. and Hatch, R. W. (1979). Effects of hatchery procedures on later return of Atlantic salmon to rivers. *Prog. Fish-Culturist* 41, 115–119.
- Huang, T. S., Ruoff, P. and Fjellidal, P. G. (2010). Diurnal expression of clock genes in pineal gland and brain and plasma levels of melatonin and cortisol in Atlantic salmon parr and smolts. *Chronobiol. Int.* 27, 1697–1714.
- Hutchison, M. J. and Iwata, M. (1998). Effect of thyroxine on the decrease of aggressive behaviour of four salmonids during the parr-smolt transformation. *Aquaculture* 168, 169–175.
- Hvidsten, N. A. and Hansen, L. P. (1988). Increased recapture rate of adult Atlantic salmon, *Salmo salar* L., stocked as smolts at high water discharge. *J. Fish Biol.* 32, 153–154.
- Iwata, M. (1995). Downstream migratory behavior of salmonids and its relationship with cortisol and thyroid hormones: A review. *Aquaculture* 135, 131–139.
- Iwata, M., Tsuboi, H., Yamashita, T., Amemiya, A., Yamada, H. and Chiba, H. (2003). Function and trigger of thyroxine surge in migrating chum salmon *Oncorhynchus keta* fry. *Aquaculture* 222, 315–329.
- Iwata, M., Yamauchi, K., Nishioka, R. S., Lin, R. and Bern, H. A. (1990). Effects of thyroxine, growth hormone and cortisol on salinity preference of juvenile coho salmon (*Oncorhynchus kisutch*). *Mar. Behav. Physiol.* 17, 191–201.
- Jarrard, H. E. (1997). Postembryonic changes in the structure of the olfactory bulb of the chinook salmon (*Oncorhynchus tshawytscha*) across its life history. *Brain, Behav. Evol.* 49, 249–260.
- Jarvi, T. (1990). Cumulative acute physiological stress in Atlantic salmon smolts: the effect of osmotic imbalance and the presence of predators. *Aquaculture* 89, 337–350.
- Johnston, C. E. and Eales, J. G. (1967). Purines in the integument of the Atlantic salmon (*Salmo salar*) during parr-smolt transformation. *J. Fish. Res. Board Can.* 24, 955–964.
- Johnston, C. E. and Saunders, R. L. (1981). Parr-smolt transformation of yearling Atlantic salmon (*Salmo salar*) at several rearing temperatures. *Can. J. Fish. Aquat. Sci.* 38, 1189–1198.
- Johnstone, K. A., Lubieniecki, K. P., Koop, B. F. and Davidson, W. S. (2011). Expression of olfactory receptors in different life stages and life histories of wild Atlantic salmon (*Salmo salar*). *Molec. Ecol.* 20, 4059–4069.
- Jonsson, B., Jonsson, N. and Hansen, L. P. (1991). Differences in life history and migratory behaviour between wild and hatchery-reared Atlantic salmon in nature. *Aquaculture* 98, 69–78.
- Kato, F. (1991). Life histories of masu and amago salmon (*Oncorhynchus masou* and *Oncorhynchus rhodurus*). In *Pacific salmon life histories* (eds. C. Groot and L. Margolis), pp. 447–520. Vancouver: UBC Press.

- Kelley, K. M., Nishioka, R. S. and Bern, H. A. (1990). In vitro effect of osmotic pressure and cortisol on prolactin cell physiology in the coho salmon (*Oncorhynchus kisutch*) during the parr-smolt transformation. *J. Exp. Zool.* 254, 72–82.
- Kiilerich, P., Kristiansen, K. and Madsen, S. S. (2007a). Cortisol regulation of ion transporter mRNA in Atlantic salmon gill and the effect of salinity on the signaling pathway. *J. Endocrinol.* 194, 417–427.
- Kiilerich, P., Kristiansen, K. and Madsen, S. S. (2007b). Hormone receptors in gills of smolting Atlantic salmon, *Salmo salar*: Expression of growth hormone, prolactin, mineralocorticoid and glucocorticoid receptors and 11 beta-hydroxysteroid dehydrogenase type 2. *Gen. Comp. Endocrinol.* 152, 295–303.
- Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F. and Mortensen, E. (2012). Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus*: a review of aspects of their life histories. *Ecol. Freshw. Fish* 12, 1–59.
- Kristinsson, J. B., Saunders, R. L. and Wiggs, A. J. (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, H. and Kawamura, H. (2002). Detection of thyroid hormone receptors in the olfactory system and brain of wild masu salmon, *Oncorhynchus masou* (Brevoort), during smolting by *in vitro* autoradiography. *Aquacult. Fish. Mngmnt* 25 (s2), 171–182.
- Langdon, J. S., Thorpe, J. E. and Roberts, R. J. (1984). Effects of cortisol and ACTH on gill Na^+/K^+ -ATPase, SDH and chloride cells in juvenile Atlantic salmon, *Salmo salar*. *Comp. Biochem. Physiol. [A]* 77, 9–12.
- Larsen, D. A., Beckman, B. R. and Dickhoff, W. W. (2001). The effect of low temperature and fasting during the winter on growth and smoltification of coho salmon. *N. Am. J. Aquacult.* 63, 1–10.
- Larsen, D. A., Swanson, P., Dickey, J. T., Rivier, J. and Dickhoff, W. W. (1998). In vitro thyrotropin-releasing activity of corticotropin-releasing hormone-family peptides in coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 109, 276–285.
- Larsen, D. A., Swanson, P. and Dickhoff, W. W. (2011). The pituitary-thyroid axis during the parr-smolt transformation of Coho salmon, *Oncorhynchus kisutch*: Quantification of TSH beta mRNA, TSH, and thyroid hormones. *Gen. Comp. Endocrinol.* 171, 367–372.
- Lawson, P. W., Logerwell, E. A., Mantua, N. J., Francis, R. C. and Agostini, V. N. (2004). Environmental factors influencing freshwater survival and smolt production in Pacific Northwest coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* 61, 360–373.
- Lema, S. C. and Nevitt, G. A. (2004). Evidence that thyroid hormone induces olfactory cellular proliferation in salmon during a sensitive period for imprinting. *J. Exp. Biol.* 207, 3317–3327.
- Leonard, J. B. K. and McCormick, S. D. (2001). Metabolic enzyme activity during smolting in stream- and hatchery-reared Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 58, 1585–1593.
- Letunic, I. and Bork, P. (2011). Interactive Tree of Life v2: online annotation and display of phylogenetic trees made easy. *Nuc. Acid Res.* 39, W475–W478.
- Levings, C. D. (1994). Feeding behaviour of juvenile salmon and significance of habitat during estuary and early sea phase. *Nord. J. Fresh. Res.* 69, 7–16.
- Li, W. M., Sorensen, P. W. and Gallaher, D. D. (1995). The olfactory system of migratory adult sea lamprey (*Petromyzon marinus*) is specifically and acutely sensitive to unique bile acids released by conspecific larvae. *J. Gen. Physiol.* 105, 569–587.
- Loge, F. J., Arkoosh, M. R., Ginn, T. R., Johnson, L. L. and Collier, T. K. (2005). Impact of environmental stressors on the dynamics of disease transmission. *Environ. Sci. Technol.* 39, 7329–7336.

- Loretz, C. A., Collie, N. L., Richman, N. H. and Bern, H. A. (1982). Osmoregulatory changes accompanying smoltification in coho salmon. *Aquaculture* 28, 67–74.
- Lorz, H. W. and McPherson, B. P. (1976). Effects of copper or zinc in fresh water on the adaptation to sea water and ATPase activity, and the effects of copper on migratory disposition of coho salmon (*Oncorhynchus kisutch*). *J. Fish. Res. Board Can.* 33, 2023–2030.
- Lubin, R. T., Rourke, A. W. and Bradley, T. M. (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., Mckinnell, S., Fangstam, H. and Berglund, I. (1994). The effect of time, size and sex on recapture rates and yield after river releases of *Salmo salar* smolts. *Aquaculture* 121, 245–257.
- MacDonald, D. J. and McKeown, B. A. (1983). The effect of Ca^{2+} levels on *in vitro* prolactin release from the rostral pars distalis of coho salmon (*Oncorhynchus kisutch*). *Can. J. Zool.* 61, 682–684.
- McCormick, S. D. (1994). Ontogeny and evolution of salinity tolerance in anadromous salmonids: Hormones and heterochrony. *Estuaries* 17, 26–33.
- McCormick, S. D. (2001). Endocrine control of osmoregulation in teleost fish. *Am. Zool.* 41, 781–794.
- McCormick, S. D., Björnsson, B. Th., Sheridan, M., Eilertson, C., Carey, J. B. and 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, S. D., Dickhoff, W. W., Duston, J., Nishioka, R. S. and Bern, H. A. (1991). Developmental differences in the responsiveness of gill Na^+, K^+ -ATPase to cortisol in salmonids. *Gen. Comp. Endocrinol.* 84, 308–317.
- McCormick, S. D., Hansen, L. P., Quinn, T. P. and Saunders, R. L. (1998). Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 55, 77–92.
- McCormick, S. D., Lerner, D. T., Monette, M. Y., Nieves-Puigdoller, K., Kelly, J. T. and Björnsson, B. Th. (2009a). Taking it with you when you go: How perturbations to the freshwater environment, including temperature, dams, and contaminants, affect marine survival of salmon. *Am. Fish. Soc. Symp.* 69, 195–214.
- McCormick, S. D., Moriyama, S. and Björnsson, B. Th. (2000). Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. *Am. J. Physiol. – Reg. Integr. Comp. Physiol.* 278, R1352–R1361.
- McCormick, S. D. and Naiman, R. J. (1984). Osmoregulation in the brook trout, *Salvelinus fontinalis*. II. Effects of size, age and photoperiod on seawater survival and ionic regulation. *Comp. Biochem. Physiol.* 79A, 17–28.
- McCormick, S. D., O’Dea, M. F., Moeckel, A. M. and Björnsson, B. Th. (2003). Endocrine and physiological changes in Atlantic salmon smolts following hatchery release. *Aquaculture* 222, 45–57.
- McCormick, S. D., Regish, A., O’Dea, M. F. and Shrimpton, J. M. (2008). Are we missing a mineralocorticoid in teleost fish? Effects of cortisol, deoxycorticosterone and aldosterone on osmoregulation, gill Na^+, K^+ -ATPase activity and isoform mRNA levels in Atlantic salmon. *Gen. Comp. Endocrinol.* 157, 35–40.
- McCormick, S. D., Regish, A. M. and Christensen, A. K. (2009b). Distinct freshwater and seawater isoforms of Na^+/K^+ -ATPase in gill chloride cells of Atlantic salmon. *J. Exp. Biol.* 212, 3994–4001.
- McCormick, S. D., Regish, A. M., Christensen, A. K., and Björnsson, B. Th. (2013a). Differential regulation of sodium-potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. *J. Exp. Biol.* in press.

- McCormick, S. D. and Saunders, R. L. (1987). Preparatory physiological adaptations for marine life in salmonids: osmoregulation, growth and metabolism. *Am. Fish. Soc. Symp.* 1, 211–229.
- McCormick, S. D., Saunders, R. L. and MacIntyre, A. D. (1989). Mitochondrial enzyme activity and ion regulation during parr-smolt transformation of Atlantic salmon (*Salmo salar*). *Fish Physiol. Biochem.* 6, 231–241.
- McCormick, S. D., Sheehan, T., Björnsson, B. Th., Lipsky, C., Kocik, J., Regish, A. M. and O’Dea, M. F. (2013b). Physiological and endocrine changes in Atlantic salmon smolts during hatchery rearing, downstream migration and ocean entry. *Can. J. Fish. Aquat. Sci.* in press.
- McCormick, S. D., Shrimpton, J. M., Moriyama, S. and Björnsson, B. Th. (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, S. D., Shrimpton, J. M., Moriyama, S. and Björnsson, B. Th. (2007). Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting. *Aquaculture* 273, 337–344.
- McCormick, S. D., Shrimpton, J. M. and Zydlewski, J. D. (1997). Temperature effects on osmoregulatory physiology of juvenile anadromous fish. In *Global Warming: Implications for Freshwater and Marine Fish* (eds. C. M. Wood and D. G. McDonald), pp. 279–301. Cambridge: Cambridge University Press.
- McGinnity, P., de Eyto, E., Cross, T. F., Coughlan, J., Whelan, K. and Ferguson, A. (2007). Population specific smolt development, migration and maturity schedules in Atlantic salmon in a natural river environment. *Aquaculture* 273, 257–268.
- Miyakoshi, Y., Nagata, M. and Kitada, S. (2001). Effect of smolt size on postrelease survival of hatchery-reared masu salmon *Oncorhynchus masou*. *Fish. Sci.* 67, 134–137.
- Mizuno, S., Misaka, N., Ando, D. and Kitamura, T. (2004). Quantitative changes of black pigmentation in the dorsal fin margin during smoltification in masu salmon, *Oncorhynchus masou*. *Aquaculture* 229, 433–450.
- Mizuno, S., Misaka, N., Ando, D., Torao, M., Urabe, H. and Kitamura, T. (2007). Effects of diets supplemented with iron citrate on some physiological parameters and on burst swimming velocity in smoltifying hatchery-reared masu salmon (*Oncorhynchus masou*). *Aquaculture* 273, 284–297.
- Mizuno, S., Ura, K., Onodera, Y., Fukada, H., Misaka, N., Hara, A., Adachi, S. and Yamauchi, K. (2001). Changes in transcript levels of gill cortisol receptor during smoltification in wild masu salmon, *Oncorhynchus masou*. *Zool. Sci.* 18, 853–860.
- Monette, M. Y. and McCormick, S. D. (2008). Impacts of short-term acid and aluminum exposure on Atlantic salmon (*Salmo salar*) physiology: a direct comparison of parr and smolts. *Aquat. Toxicol.* 86, 216–226.
- Morin, P. P., Andersen, O., Haug, E. and Doving, K. B. (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, P. P. and Doving, K. B. (1992). Changes in the olfactory function of Atlantic salmon, *Salmo salar*, in the course of smoltification. *Can. J. Fish. Aquat. Sci.* 49, 1704–1713.
- Morin, P. P., Hara, T. J. and Eales, J. G. (1995). T-4 depresses olfactory responses to L-alanine and plasma T-3 and T-3 production in smoltifying Atlantic salmon. *Am. J. Physiol – Regul. Integr. C* 38, R1434–R1440.
- Moriyama, S., Ito, T., Takahashi, A., Amano, M., Sower, S. A., Hirano, T., Yamamori, K. and Kawauchi, H. (2002). A homolog of mammalian PRL-releasing peptide (fish arginyl-

- phenylalanyl-amide peptide) is a major hypothalamic peptide of PRL release in teleost fish. *Endocrinol.* 143, 2071–2079.
- Nevitt, G. A., Dittman, A. H., Quinn, T. P. and Moody, W. J. (1994). Evidence for a peripheral olfactory memory in imprinted salmon. *Proc. Natl Acad. Sci. U. S. A.* 91, 4288–4292.
- Nielsen, C., Madsen, S. S. and Björnsson, B. Th. (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, T. O., Ebbesson, L. E., Stefansson, S. O., Madsen, S. S., McCormick, S. D., Björnsson, B. Th. and Prunet, P. (2007). Differential expression of gill Na^+, K^+ -ATPase α - and β -subunits, $\text{Na}^+, \text{K}^+, 2\text{Cl}^-$ cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon *Salmo salar*. *J. Exp. Biol.* 210, 2885–2896.
- Nilsen, T. O., Ebbesson, L. O. E., Küllerich, P., Björnsson, B. Th., Madsen, S. S., McCormick, S. D. and Stefansson, S. O. (2008). Endocrine systems in juvenile anadromous and landlocked Atlantic salmon (*Salmo salar*): Seasonal development and seawater acclimation. *Gen. Comp. Endocrinol.* 155, 762–772.
- Nishioka, R. S., Bern, H. A., Lai, K. V., Nagahama, Y. and Grau, E. G. (1982). Changes in the endocrine organs of coho salmon during normal and abnormal smoltification – an electron-microscopy study. *Aquaculture* 28, 21–38.
- Nordeng, H. (1977). A pheromone hypothesis for homeward migration in anadromous salmonids. *Oikos* 28, 155–159.
- Ogata, H. Y., Konno, S. and Silverstein, J. T. (1998). Muscular buffering capacity of the parr and smolts in *Oncorhynchus masou*. *Aquaculture* 168, 303–310.
- Ojima, D. and Iwata, M. (2007a). Seasonal changes in plasma thyroxine kinetics in coho salmon *Oncorhynchus kisutch* during smoltification. *Aquaculture* 273, 329–336.
- Ojima, D. and Iwata, M. (2007b). The relationship between thyroxine surge and onset of downstream migration in chum salmon *Oncorhynchus keta* fry. *Aquaculture* 273, 185–193.
- Ojima, D. and Iwata, M. (2009). Central administration of growth hormone-releasing hormone triggers downstream movement and schooling behavior of chum salmon (*Oncorhynchus keta*) fry in an artificial stream. *Comp. Biochem. Physiol. A – Molecul. Integr. Physiol.* 152, 293–298.
- Ojima, D. and Iwata, M. (2010). Central administration of growth hormone-releasing hormone and corticotropin-releasing hormone stimulate downstream movement and thyroxine secretion in fall-smolting coho salmon (*Oncorhynchus kisutch*). *Gen. Comp. Endocrinol.* 168, 82–87.
- Orciari, R. D. and Leonard, G. H. (1996). Length characteristics of smolts and timing of downstream migration among three strains of Atlantic salmon in a southern New England stream. *N. Am. J. Fish. Manag.* 16, 851–860.
- Parhar, I. S. and 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.
- Parry, G. (1960). The development of salinity tolerance in the salmon, *Salmo salar* (L.) and some related species. *J. Exp. Biol.* 37, 425–434.
- Patino, R., Schreck, C. B. and Redding, J. M. (1985). Clearance of plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. *Comp. Biochem. Physiol. [A]* 82A, 531–535.
- Peake, S. and McKinley, R. S. (1998). A re-evaluation of swimming performance in juvenile salmonids relative to downstream migration. *Can. J. Fish. Aquat. Sci.* 55, 682–687.
- Pelis, R. M. and McCormick, S. D. (2001). Effects of growth hormone and cortisol on $\text{Na}^+, \text{K}^+, 2\text{Cl}^-$ cotransporter localization and abundance in the gills of Atlantic salmon. *Gen. Comp. Endocrinol.* 124, 134–143.

- Pelis, R. M. and McCormick, S. D. (2003). Fin development in stream- and hatchery-reared Atlantic salmon. *Aquaculture* 220, 525–536.
- Pelis, R. M., Zydlewski, J. and McCormick, S. D. (2001). Gill $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransporter abundance and location in Atlantic salmon: effects of seawater and smolting. *Am. J. Physiol. – Reg. Integr. & Comp. Physiol.* 280, R1844–R1852.
- Pisam, M., Prunet, P., Boeuf, G. and 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.
- Price, C. S. and Schreck, C. B. (2003). Stress and saltwater-entry behavior of juvenile chinook salmon (*Oncorhynchus tshawytscha*): conflicts in physiological motivation. *Can. J. Fish. Aquat. Sci.* 60, 910–918.
- Primmitt, D. R. N., Eddy, F. B., Miles, M. S., Talbot, C. and Thorpe, J. E. (1988). Transepithelial ion exchange in smolting Atlantic salmon (*Salmo salar* L.). *Fish Physiol. Biochem.* 5, 181–186.
- Prunet, P., Boeuf, G., Bolton, J. P. and Young, G. (1989). Smoltification and seawater adaptation in Atlantic salmon (*Salmo salar*): plasma prolactin, growth hormone, and thyroid hormones. *Gen. Comp. Endocrinol.* 74, 355–364.
- Quinn, M. C. J., Veillette, P. A. and Young, G. (2003). Pseudobranch and gill Na^+ , K^+ -ATPase activity in juvenile chinook salmon, *Oncorhynchus tshawytscha*: developmental changes and effects of growth hormone, cortisol and seawater transfer. *Comp. Biochem. Physiol. A, Mol. Integr. Physiol.* 135, 249–262.
- Quinn, T. P., Brannon, E. L. and Dittman, A. H. (1989). Spatial aspects of imprinting and homing in coho salmon. *Fish. Bull.* 87, 769–774.
- Rechisky, E. L., Welch, D. W., Porter, A. D., Jacobs, M. C. and Ladouceur, A. (2009). Experimental measurement of hydrosystem-induced delayed mortality in juvenile Snake River spring Chinook salmon (*Oncorhynchus tshawytscha*) using a large-scale acoustic array. *Can. J. Fish. Aquat. Sci.* 66, 1019–1024.
- Redding, J. M., Patino, R. and Schreck, C. B. (1984). Clearance of corticosteroids in yearling coho salmon, *Oncorhynchus kisutch*, in fresh water and seawater and after stress. *Gen. Comp. Endocrinol.* 54, 433–443.
- Richards, J. G., Semple, J. W., Bystriansky, J. S. and Schulte, P. M. (2003). Na^+/K^+ -ATPase α -isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. *J. Exp. Biol.* 206, 4475–4486.
- Richman, N. H., Tai de Diaz, S., Nishioka, R. S., Prunet, P. and Bern, H. A. (1987). Osmoregulatory and endocrine relationships with chloride cell morphology and density during smoltification in coho salmon (*Oncorhynchus kisutch*). *Aquaculture* 60, 265–285.
- Rimmer, D. M., Paim, U. and Saunders, R. L. (1983). Autumnal habitat shift in juvenile Atlantic salmon (*Salmo salar*) in a small river. *Can. J. Fish. Aquat. Sci.* 40, 671–680.
- Robertson, L. S. and McCormick, S. D. (2012). Transcriptional profiling of the parr-smolt transformation in Atlantic salmon. *Comp. Biochem. Physiol. A – Molecul. Integr. Physiol.* 7, 351–360.
- Rosseland, B. O., Kroglund, F., Staurnes, M., Hindar, K. and Kvellestad, A. (2001). Tolerance to acid water among strains and life stages of Atlantic salmon (*Salmo salar* L.). *Water Air Soil. Pollut.* 130, 899–904.
- Rounsefell, G. A. (1958). Anadromy in North American salmonidae. *Fish. Bull.* 58, 171–185.
- Sakamoto, T., Hirano, T., Madsen, S. S., Nishioka, R. S. and Bern, H. A. (1995). Insulin-like growth factor I gene expression during parr-smolt transformation of coho salmon. *Zool. Sci.* 12, 249–252.

- Saloniemi, I., Jokikokko, E., Kallio-Nyberg, I., Jutila, E. and Pasanen, P. (2004). Survival of reared and wild Atlantic salmon smolts: size matters more in bad years. *ICES J. Mar. Sci.* 61, 782–787.
- Schreck, C. B., Solazzi, M. F., Johnson, S. L. and Nickelson, T. E. (1989). Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch*. *Aquaculture* 82, 15–20.
- Schreck, C. B., Stahl, T. P., Davis, L. E., Roby, D. D. and Clemens, B. J. (2006). Mortality estimates of juvenile spring-summer Chinook salmon in the Lower Columbia River and estuary, 1992–1998: Evidence for delayed mortality? *Trans. Am. Fish. Soc.* 135, 457–475.
- Seidelin, M. and Madsen, S. S. (1997). Prolactin antagonizes the seawater-adaptive effect of cortisol and growth hormone in anadromous brown trout (*Salmo trutta*). *Zool. Sci.* 14, 249–256.
- Seidelin, M. and Madsen, S. S. (1999). Endocrine control of Na⁺,K⁺-ATPase and chloride cell development in brown trout (*Salmo trutta*): interaction of insulin-like growth factor-I with prolactin and growth hormone. *J. Endocr.* 162, 127–135.
- Sheridan, M. (1986). Effects of thyroxine, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. *Gen. Comp. Endocrinol.* 64, 220–238.
- Shimizu, M., Kudo, H., Ueda, H., Hara, A., Shimazaki, K. and Yamauchi, K. (1993). Identification and immunological properties of an olfactory system-specific protein in kokanee salmon (*Oncorhynchus nerka*). *Zool. Sci.* 10, 287–294.
- Shoji, T., Ueda, H., Ohgami, T., Sakamoto, T., Katsuragi, Y., Yamauchi, K. and Kurihara, K. (2000). Amino acids dissolved in stream water as possible home stream odorants for masu salmon. *Chem. Sens.* 25, 533–540.
- Shoji, T., Yamamoto, Y., Nishikawa, D., Kurihara, K. and Ueda, H. (2003). Amino acids in stream water are essential for salmon homing and migration. *Fish Physiol. Biochem.* 28, 249–251.
- Shrimpton, J. M., Bernier, N. J., Iwama, G. K. and Randall, D. J. (1994). Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) before and after saltwater exposure. *Can. J. Fish. Aquat. Sci.* 51, 2170–2178.
- Shrimpton, J. M., Devlin, R. H., Mclean, E., Byatt, J. C., Donaldson, E. M. and Randall, D. J. (1995). Increases in gill corticosteroid receptor abundance and saltwater tolerance in juvenile coho salmon (*Oncorhynchus kisutch*) treated with growth hormone and placental lactogen. *Gen. Comp. Endocrinol.* 98, 1–15.
- Shrimpton, J. M. and McCormick, S. D. (1998a). Regulation of gill cytosolic corticosteroid receptors in juvenile Atlantic salmon: Interaction effects of growth hormone with prolactin and triiodothyronine. *Gen. Comp. Endocrinol.* 112, 262–274.
- Shrimpton, J. M. and McCormick, S. D. (1998b). Seasonal differences in plasma cortisol and gill corticosteroid receptors in upper and lower mode juvenile Atlantic salmon. *Aquaculture* 168, 205–219.
- Shrimpton, J. M. and Randall, D. J. (1994). Downregulation of corticosteroid receptors in gills of coho salmon due to stress and cortisol treatment. *Am. J. Physiol.* 267, R432–R438.
- Sigholt, T., Asgard, T. and 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, T. D., Clements, K. M., Semple, J. W., Schulte, P. M., Bystriansky, J. S., Finstad, B., Fleming, I. A. and McKinley, R. S. (2002). Seawater tolerance and gene expression in two strains of Atlantic salmon smolts. *Can. J. Fish. Aquat. Sci.* 59, 125–135.

- Solbakken, V. A., Hansen, T. and Stefansson, S. O. (1994). Effects of photoperiod and temperature on growth and parr-smolt transformation in Atlantic salmon (*Salmo salar* L) and subsequent performance in seawater. *Aquaculture* 121, 13–27.
- Specker, J. L. (1982). Interrenal function and smoltification. *Aquaculture* 28, 59–66.
- Specker, J. L., Brown, C. L. and Bern, H. A. (1992). Asynchrony of changes in tissue and plasma thyroid hormones during the parr-smolt transformation of coho salmon. *Gen. Comp. Endocrinol.* 88, 397–405.
- Specker, J. L., Distefano, J. J., Grau, E. G., Nishioka, R. S. and Bern, H. A. (1984). Development-associated changes in thyroxine kinetics in juvenile salmon. *Endocrinol.* 115, 399–406.
- Specker, J. L. and Schreck, C. B. (1982). Changes in plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 46, 53–58.
- Specker, J. L. and Schreck, C. B. (1984). Thyroidal response to mammalian thyrotropin during smoltification of juvenile coho salmon (*Oncorhynchus kisutch*). *Comp. Biochem. Physiol.* 78, 441–444.
- Staurnes, M., Lysfjord, G., Hansen, L. P. and Heggberget, T. G. (1993). Recapture rates of hatchery-reared Atlantic salmon (*Salmo salar*) related to smolt development and time of release. *Aquaculture* 118, 327–337.
- Stefansson, S. O., Björnsson, B. Th., Ebbesson, L. O. E. and McCormick, S. D. (2008). Smoltification. In *Fish Larval Physiology*. (eds. R. N. Finn and B. G. Kapoor), pp. 639–681. Enfield, NH, USA: Science Publishers.
- Stefansson, S. O., Björnsson, B. Th., Sundell, K., Nyhammer, G. and McCormick, S. D. (2003). Physiological characteristics of wild Atlantic salmon post-smolts during estuarine and coastal migration. *J. Fish Biol.* 63, 942–955.
- Stefansson, S. O., Haugland, M., Björnsson, B. Th., McCormick, S. D., Holm, M., Ebbesson, L. O. E., Holst, J. C. and Nilsen, T. O. (2012). Growth, osmoregulation and endocrine changes in wild Atlantic salmon smolts and post-smolts during marine migration. *Aquaculture* 363, 127–136.
- Stefansson, S. O., Nilsen, T. O., Ebbesson, L. O. E., Wargelius, A., Madsen, S. S., Björnsson, B. Th. and McCormick, S. D. (2007). Molecular mechanisms of continuous light inhibition of Atlantic salmon parr-smolt transformation. *Aquaculture* 273, 235–245.
- Sundell, K., Jutfelt, F., Agustsson, T., Olsen, R. E., Sandblom, E., Hansen, T. and Björnsson, B. Th. (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.
- Sykes, G. E., Johnson, C. J. and Shrimpton, J. M. (2009). Temperature and flow effects on migration timing of chinook salmon smolts. *Trans. Am. Fish. Soc.* 138, 1252–1265.
- Sykes, G. E. and Shrimpton, J. M. (2010). Effect of temperature and current manipulation on smolting in Chinook salmon (*Oncorhynchus tshawytscha*): the relationship between migratory behaviour and physiological development. *Can. J. Fish. Aquat. Sci.* 67, 191–201.
- Takei, Y. and McCormick, S. D. (2013). Hormonal control of fish euryhalinity. In *Fish Physiology*, Vol. 32, *Euryhaline Fishes* (eds. S. D. McCormick, C. J. Brauner and A. P. Farrell), pp. 69–124. Amsterdam: Academic Press.
- Taylor, E. B. (1990). Phenotypic correlates of life-history variation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. *J. Anim. Ecol.* 59, 455–468.
- Thorpe, J. E. (1984). Downstream movements of juvenile salmonids: a forward speculative view. In *Mechanisms of migration in fishes* (eds. J. D. McCleave, G. P. Arnold, J. J. Dodson and W. H. Neill), pp. 387–396. New York: Plenum Press.
- Thorpe, J. E. (1994a). An alternative view of smolting in salmonids. *Aquaculture* 121, 105–113.
- Thorpe, J. E. (1994b). Salmonid fishes and the estuarine environment. *Estuaries* 17, 76–93.
- Thorpe, J. E. and Morgan, R. I. G. (1978). Periodicity in Atlantic salmon, *Salmo salar*, smolt migration. *J. Fish Biol.* 12, 541–548.

- Thorstad, E. B., Whoriskey, F., Uglem, I., Moore, A., Rikardsen, A. H. and Finstad, B. (2012). A critical life stage of the Atlantic salmon *Salmo salar*: behaviour and survival during the smolt and initial post-smolt migration. *J. Fish Biol.* 81, 500–542.
- Tipsmark, C. K., Jorgensen, C., Brande-Lavridsen, N., Engelund, M., Olesen, J. H. and Madsen, S. S. (2009). Effects of cortisol, growth hormone and prolactin on gill claudin expression in Atlantic salmon. *Gen. Comp. Endocrinol.* 163, 270–277.
- Tipsmark, C. K., Kiillerich, P., Nilsen, T. O., Ebbesson, L. O. E., Stefansson, S. O. and Madsen, S. S. (2008). Branchial expression patterns of claudin isoforms in Atlantic salmon during seawater acclimation and smoltification. *Am. J. Physiol. – Reg. Integr. Comp. Physiol.* 294, R1563–R1574.
- Tipsmark, C. K. and Madsen, S. S. (2009). Distinct hormonal regulation of Na⁺,K⁺-ATPase genes in the gill of Atlantic salmon (*Salmo salar* L.). *J. Endocrinol.* 203, 301–310.
- Tipsmark, C. K., Mahmmoud, Y. A., Borski, R. J. and Madsen, S. S. (2010a). FXYD-11 associates with Na⁺K⁺ATPase in the gill of Atlantic salmon: regulation and localization in relation to changed ion-regulatory status. *Am. J. Physiol. – Reg. Integr. Comp. Physiol.* 299, R1212–R1223.
- Tipsmark, C. K., Sorensen, K. J., Hulgard, K. and Madsen, S. S. (2010b). Claudin-15 and -25b expression in the intestinal tract of Atlantic salmon in response to seawater acclimation, smoltification and hormone treatment. *Comp. Biochem. Physiol. A – Mol. Integr. Physiol.* 155, 361–370.
- Tipsmark, C. K., Sorensen, K. J. and Madsen, S. S. (2010c). Aquaporin expression dynamics in osmoregulatory tissues of Atlantic salmon during smoltification and seawater acclimation. *J. Exp. Biol.* 213, 368–379.
- Tucker, S., Trudel, M. and Welch, D. W. (2012). Annual coastal migration of juvenile Chinook salmon: static stock-specific patterns in a highly dynamic ocean. *Mar. Ecol. Prog. Ser.* 449, 245–284.
- Uchida, K., Kaneko, T., Tagawa, M. and Hirano, T. (1998). Localization of cortisol receptor in branchial chloride cells in chum salmon fry. *Gen. Comp. Endocrinol.* 109, 175–185.
- Ueda, H. (2011). Physiological mechanism of homing migration in Pacific salmon from behavioral to molecular biological approaches. *Gen. Comp. Endocrinol.* 170, 222–232.
- Veillette, P. A., Sundell, K. and Specker, J. L. (1995). Cortisol mediates the increase in intestinal fluid absorption in Atlantic salmon during parr smolt transformation. *Gen. Comp. Endocrinol.* 97, 250–258.
- Veillette, P. A., White, R. J. and Specker, J. L. (1993). Changes in intestinal fluid transport in Atlantic salmon (*Salmo salar* L) during parr-smolt transformation. *Fish. Physiol. Biochem.* 12, 193–202.
- Veillette, P. A. and Young, G. (2004). Temporal changes in intestinal Na⁺, K⁺-ATPase activity and in vitro responsiveness to cortisol in juvenile chinook salmon. *Comp. Biochem. Physiol. A – Mol. Integr. Physiol.* 138, 297–303.
- Virtanen, E., Soderholmtna, L., Soivio, A., Forsman, L. and Muona, M. (1991). Effect of physiological condition and smoltification status at smolt release on subsequent catches of adult salmon. *Aquaculture* 97, 231–257.
- Wagner, H. H. (1974). Photoperiod and temperature regulation of smolting in steelhead trout. *Can. J. Zool.* 52, 219–234.
- Wagner, H. H., Conte, F. P. and Fessler, J. L. (1969). Development of osmotic and ionic regulation in two races of chinook salmon *O. tshawytscha*. *Comp. Biochem. Physiol.* 29, 325–341.
- Welch, D. W., Melnychuk, M. C., Payne, J. C., Rechisky, E. L., Porter, A. D., Jackson, G. D., Ward, B. R., Vincent, S. P., Wood, C. C. and Semmens, J. (2011). In situ measurement of coastal ocean movements and survival of juvenile Pacific salmon. *PNAS* 108, 8708–8713.

- Wilson, M. V. H. and Williams, R. R. G. (2010). Salmoniform fishes: key fossils, supertree, and possible morphological synapomorphies. In *Origin and Phylogenetic Interrelationships of Teleosts* (eds. J. S. Nelson, H.-P. Schultze and M. V.H. Wilson), pp. 379–409. Munchen: Verlag Dr. Friedrich Pfeil.
- Wood, A. W., Duan, C. M. and Bern, H. A. (2005). Insulin-like growth factor signaling in fish. *Int. Rev. Cytol.* 243, 215–285.
- Yada, T., Takahashi, K. and Hirano, T. (1991). Seasonal changes in seawater adaptability and plasma levels of prolactin and growth hormone in landlocked sockeye salmon (*Oncorhynchus nerka*) and amago salmon (*O. rhodurus*). *Gen. Comp. Endocrinol.* 82, 33–44.
- Yamamoto, Y., Hino, H. and Ueda, H. (2010). Olfactory imprinting of amino acids in lacustrine sockeye salmon. *PLOS ONE* 5, e8633.
- Yeoh, C.-G., Kerstetter, T. H. and Loudenslager, E. J. (1991). Twenty-four hour seawater challenge test for coastal cutthroat trout. *Progr. Fish-Culturist* 53, 173–176.
- Young, G. (1988). Enhanced response of the interrenal of coho salmon (*Oncorhynchus kisutch*) to ACTH after growth hormone treatment in vivo and in vitro. *Gen. Comp. Endocrinol.* 71, 85–92.
- Young, G. (1989). Cortisol secretion in vitro by the interrenal of coho salmon (*Oncorhynchus kisutch*) during smoltification: relationship with plasma thyroxine and plasma cortisol. *Gen. Comp. Endocrinol.* 63, 191–200.
- Young, G., Björnsson, B. Th., Prunet, P., Lin, R. J. and Bern, H. A. (1989). Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, growth hormone, thyroid hormones, and cortisol. *Gen. Comp. Endocrinol.* 74, 335–345.
- Youngson, A. F., Hansen, L. P., Jonsson, B. and Naesje, T. F. (1989). Effects of exogenous thyroxine or prior exposure to raised water-flow on the downstream movement of hatchery-reared Atlantic salmon smolts. *J. Fish Biol.* 34, 791–797.
- Zaugg, W. S. and McLain, L. R. (1970). Adenosine triphosphatase activity in gills of salmonids: seasonal variations and salt water influences in coho salmon, *Oncorhynchus kisutch*. *Comp. Biochem. Physiol.* 35, 587–596.
- Zaugg, W. S. and McLain, L. R. (1976). Influence of water temperature on gill sodium, potassium-stimulated ATPase activity in juvenile coho salmon (*Oncorhynchus kisutch*). *Comp. Biochem. Physiol.* 54A, 419–421.
- Zydlewski, G. B., Haro, A. and McCormick, S. D. (2005). Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts. *Can. J. Fish. Aquat. Sci.* 62, 68–78.