

Chapter 1

Salmonid Reproductive Migration and Effects on Sexual Maturation

Kazufumi Hayashida, Kosuke Fukaya, Arjan P. Palstra
and Hiroshi Ueda

Abstract Salmon has amazing abilities to migrate long distances from the ocean to their natal streams for reproduction. Reproductive homing migration was investigated by two different research approaches from behavioural biology to endocrinology using chum salmon in the north Pacific Ocean as well as two land-locked salmon species. These species are lacustrine sockeye and masu salmon in Lake Toya and Lake Shikotsu, Hokkaido, Japan where the lakes serve as a model “ocean”. Physiological biotelemetry techniques were applied to examine the homing behaviours of adult chum salmon from the Bering Sea to Hokkaido as well as lacustrine sockeye and masu salmon in Lake Toya, and revealed that salmon can navigate in open water using different sensory systems. The hormone profiles in

Wageningen Aquaculture is a consortium of Institute for Marine Resources and Ecosystem Studies (IMARES) and Aquaculture and Fisheries Group (AFI, Wageningen University), both part of Wageningen University and Research Centre (WUR).

K. Hayashida · K. Fukaya · H. Ueda
Division of Biosphere Science, Graduate School of Environmental Science,
Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

K. Hayashida
Watershed Environmental Engineering Research Team, Civil Engineering Research Institute
for Cold Region, Sapporo, Hokkaido 062-8602, Japan

A. P. Palstra
Institute for Marine Resources and Ecosystem Studies (IMARES), Wageningen
Aquaculture, Wageningen University and Research Center, Korringaweg 5,
4401 Yerseke, NT, The Netherlands

H. Ueda (✉)
Laboratory of Aquatic Bioresources and Ecosystem, Section of Ecosystem Conservation,
Field Science Center for Northern Biosphere, Hokkaido University, Sapporo,
Hokkaido 060-0809, Japan
e-mail: hueda@fsc.hokudai.ac.jp

the brain–pituitary–gonadal (BPG) axis were investigated in chum salmon and lacustrine sockeye salmon during their homing migration, and clarified that salmon gonadotropin-releasing hormone (sGnRH) plays leading roles on homing migration. The interaction between metabolism and sexual maturation under exercise, as experienced during the salmonid anorexic reproductive migration, has been studied by swimming the facultative migrant rainbow trout in a large swim flume. RNA sequencing of the red and white muscle transcriptome and microarray analysis of the ovary has been performed to identify hormonal and metabolic actors that are important in switching from the growth phase to the migration phenotype. Olfaction of spawning ground specific smell is suggested as switch from the navigating migration to the sexual maturation phenotype.

1.1 Introduction

Salmonids have an amazing ability to migrate thousands of kilometres from the sea to their natal river for reproduction. The reproductive homing migration is one of the most interesting aspects of the salmon life cycle and most challenging to study. It is now widely accepted that some specific odorant factors of the natal river are imprinted in particular areas of the nervous systems of juvenile salmon during downstream migration, and that adult salmon evokes these factors to recognise the natal river during the homing migration (Ueda 2011). However, there are still many unknowns regarding which sensory systems play leading roles in open water orientation and which hormones control homing behaviour. Moreover, in order to calculate the time needed to move from the feeding habitat to the natal river as well as from the river mouth to the spawning area, salmon must have an accurate biological clock. There are four Pacific salmon in Japan: pink salmon (*Oncorhynchus gorbuscha* Walbaum), chum salmon (*O. keta* Walbaum), sockeye salmon (*O. nerka* Walbaum) and masu salmon (*O. masou* Brevoort). Life cycles of these species are quite different between the former two and the latter two fishes (Fig. 1.1). All juvenile fries of pink and chum salmon migrate downstream within a few months after emergence, and adult fishes migrate upstream within a few weeks before final gonadal maturation. On the other hand, smolts of sockeye and masu salmon stay for one and a half year in streams or lakes, conduct downstream migration while obtaining seawater adaptability, and adult fishes perform upstream migration at least several months before reproduction. There are also land-locked forms like lacustrine sockeye and masu salmon, which offer good systems for studying sexual maturation during reproductive migration in Lake Toya and Shikotsu in Hokkaido where the lakes serve as a model “ocean”. Three different physiological approaches have been conducted; (1) behavioural studies using biotelemetry techniques, (2) endocrinological studies on the BPG axis of migrating salmonid species in the field and (3) simulated long-term migration under laboratory conditions.

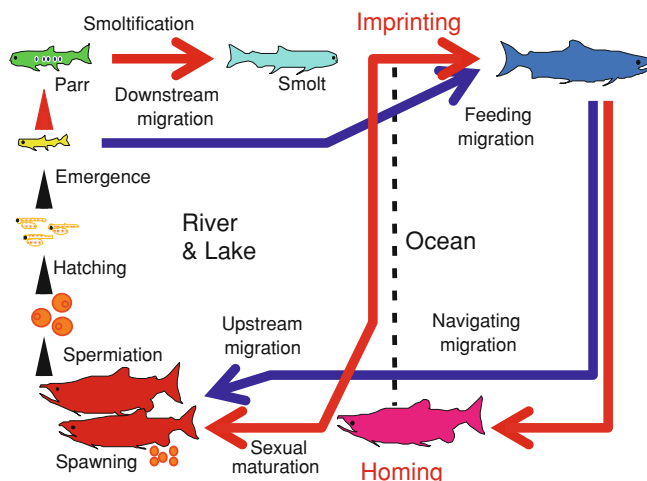


Fig. 1.1 Life cycle of two different types of Pacific salmonid species in Japan. *Blue line* chum salmon; *Red line* anadromous and lacustrine sockeye and masu salmon. There are four different migrations (downstream, feeding, navigating and upstream migration) in relation to imprinting and homing to the natal stream. All juvenile fries of chum salmon migrate downstream within a few months after emergence, and adult fishes migrate upstream within a few weeks before sexual maturation. In contrast, smolts of sockeye and masu salmon stay for one and half year in streams or lakes, conduct downstream migration while obtaining seawater adaptability, and adult fishes perform upstream migration at least several months before reproduction. There are also land-locked forms like lacustrine sockeye and masu salmon

1.2 Behavioural Study

For open water orientation and navigation mechanism of salmon, the contributions of a map and compass system, and rheotaxis to oceanic currents have been discussed (Dittman and Quinn 1996; Quinn 2005). The recent rapid development in biotelemetry techniques, such as those of ultrasonic and/or radio telemetry, data logging and/or pop-up telemetry and satellite telemetry, makes it possible to investigate wild animal behaviour on land or in the ocean. Underwater behaviour of salmon in open water which was impossible to monitor by previous techniques can now be analysed in detail using these new techniques, especially satellite telemetry (Cooke et al. 2004; Ueda 2004).

1.2.1 Chum Salmon from the Bering Sea to Hokkaido, Japan

Chum salmon caught by longline in June, 2000 in the central Bering Sea (56°30'N, 179°00'E) in a healthy condition was determined to have a Japanese origin by scale analysis. A propeller data logger, which recorded swimming speed (5 s sampling), depth (5 s sampling) and temperature (1 min sampling), was

attached externally in the dorsal musculature of the fish anterior to the dorsal fin (Tanaka et al. 2005). We released 27 chum salmon with data loggers, and retrieved one data logger on September, 2000 from a set net on the east coast of Hokkaido, Japan (43°20'N, 145°46'E). The first record of swimming profiles of homing chum salmon in the oceanic phase for 67 days in the straight distance of 2,750 km revealed that average swimming speed, depth and temperature were 62 ± 12 cm/sec, 10.4 ± 14.7 m, and 9.2 ± 0.2 °C, respectively. Both swimming speed and depth had two peaks around the dawn and sunset with a small peak around the midnight. The fish showed sequential up-and-down movements near the thermocline during the twilight and the daytime. These diurnal patterns suggest that the homing chum salmon allocated its time for foraging and the foraging strategy differed between the daytime and nighttime. These results indicate that the homing chum salmon had navigation abilities in its homeward direction and that current transport may have assisted the successful migration. During the accurate homing migration in open water, salmon must recognise exact locations (map) and compass direction (orientation), and must have a biological clock. However, for sea-run anadromous populations, it is difficult to carry out physiologically controlled and manipulated experiments as fish move from the sea in their prematuration phase to their natal stream where they become mature. In contrast, lacustrine salmon populations offer a good model system for studying homing behaviours from open water to the natal area for reproduction.

1.2.2 Lacustrine Sockeye and Masu Salmon in Lake Toya, Hokkaido, Japan

Lake Toya (surface area 71 km², average and maximum depth 116 m and 179 m, respectively) is a large caldera lake in Hokkaido, Japan. The homing migrations of mature lacustrine sockeye salmon, whose sensory cues were impaired, were tracked from the centre of the lake to the natal area using the ultrasonic tracking system (Ueda et al. 1998). Both a mature male sockeye salmon with attached control brass ring and a mature male whose magnetic cues were interfered with magnetic ring returned straight to the natal area after 1 h of random movement. A mature male sockeye salmon whose visual and magnetic cues were both blocked, moved in a direction opposite to the natal area and was rediscovered in the natal area on the following evening, suggesting the possibility of involvement of olfactory cues in finding the natal area. A blinded male sockeye salmon was also moved to the shore of Naka-Toya far from the natal area in the evening, and stayed for a few days. The homing migrations of mature lacustrine masu salmon were also tracked in Lake Toya (Ueda et al. 2000). A mature control male masu salmon moved constantly along the coast and stopped his movement at the mouth of river. A blinded mature female masu salmon was released and moved randomly away from the coast. A mature male masu salmon whose olfactory cue was blocked moved randomly along the coast, and then tended to move away from the coast.

The ultrasonic location transmitters were combined with sensory ablation to evaluate homing capability, particularly orientation ability, of sockeye and masu salmon. Using this method, visual cues appeared to be critical to the straight homing of sockeye salmon, while magnetic cues did not appear to be necessary for successful return to the natal area. However, magnetoreceptor cells have been identified in the nose of rainbow trout (*O. mykiss* Walbaum) (Walker et al. 1997). Further study should be done to investigate the involvement of magnetic cues in salmon homing migration. It is quite interesting to compare the straight movements of sockeye salmon with the coastal movement behaviours of masu salmon (Ueda 2004). These two species show large differences in ocean distribution. Sockeye salmon distribute widely in the North Pacific Ocean, while masu salmon are narrowly distributed in the west North Pacific Ocean (Kaeriyama and Ueda 1998). These data suggest some evolutionary aspects of successful homing migration of salmonids where the narrowly distributed masu salmon only need coastal recognition ability, but widely distributed sockeye salmon must obtain open water cues for orientation.

1.3 Endocrinological Study

The salmon homing migration is closely related to gonadal maturation, which is regulated mainly by the BPG axis. Two molecular types of gonadotropin-releasing hormone (GnRH), sGnRH and chicken GnRH-II (cGnRH-II) exist in various brain regions of salmonids (Amano et al. 1977). In particular, sGnRH in the olfactory system, the terminal nerve and the preoptic area are considered to play leading roles in salmon homing migration. sGnRH in the preoptic area controls gonadotropin (GTH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) synthesis and release from the pituitary gland. GTHs induce steroidogenesis in the gonads, and steroid hormones stimulate gametogenesis and final gameto-maturation; estradiol- 17β (E_2) and testosterone (T) are active in vitellogenesis, T and 11-ketotestosterone (11KT) in spermatogenesis and $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (DHP) in final gameto-maturation in both sexes (Nagahama 1999). It is very important to investigate hormone profiles in the BPG axis of salmon during homing migration as well as gonadal maturation (Ueda and Yamauchi 1995; Ueda 1999) to clarify how these hormones control/mediate homing migration and gonadal maturation.

1.3.1 Hormone Profiles of Chum Salmon During Reproductive Migration

The hormone profiles in the BPG axis of chum salmon migrating from the Bering Sea to the spawning ground in the Chitose River, Hokkaido, Japan, in 2001 were measured using specific time-resolved fluoroimmunoassay (TR-FIA) systems

(Yamada et al. 2002). The level of sGnRH in the olfactory bulb (OB) of both sexes showed a peak from the coastal sea to the river mouth of the Ishikari River, where the olfactory discriminating ability of the natal stream should be functioning and also in the telencephalon (TC), where it increased at the branch point of the Chitose River from the Ishikari River where the olfactory functions should also be highly activated. In the pituitary gland, sGnRH levels tended to increase at the same time as elevation in LH levels from the coastal sea in females to the river mouth of the Ishikari River in males. In contrast, FSH levels did not show any clear correlation with sGnRH levels in the pituitary gland. Although the roles of cGnRH-II in these brain regions remain to be elucidated, the levels of cGnRH-II in the medulla oblongata (MO) increased in both sexes at the prespawning ground while that in the optic tectum (OT) also increased in males. In the diencephalon (DC) and cerebellum (CB), cGnRH-II levels showed no significant changes during homing migration.

Serum steroid hormone levels showed similar profiles as previous observations (Ueda et al. 1984; Ueda 1999); E₂ in females and 11KT in males increased during vitellogenesis and spermatogenesis, respectively, and DHP increased dramatically at the time of final gonadal maturation in both sexes. It is quite interesting to note that both sGnRH levels in the TC and serum T levels in both sexes showed a coincident peak at the branch point of the Chitose River from the Ishikari River. These results confirm previous findings that sGnRH plays a role in GTH secretion in the pituitary of chum salmon, and sGnRH and cGnRH-II might be involved in brain region-dependent roles on gonadal maturation and homing migration of salmon.

1.3.2 Homing Profiles and Hormonal Manipulation in Lacustrine Sockeye Salmon

Since it is difficult to carry out experimental treatments to manipulate endocrinological functions in sea-run anadromous salmon owing to the salinity differences between seawater and freshwater, lacustrine salmon populations also offer a good model system for studying hormonal controlling mechanisms of salmon homing. In Lake Shikotsu (surface area 78 km², average and maximum depth 265 m and 363 m, respectively), adult sockeye salmon were captured from September to November adjacent to their natal hatchery prior to spawning. They were sampled for serum steroid hormones, tagged, and released in the centre of the lake. Fishes were sampled again at recapture to characterise changes in steroid hormone levels in individual migrants as well as homing duration and percentage in each month (Sato et al. 1997). Homing duration was significantly shortened from September to October in males and from October to November in females. All males returned faster than females early in September and October, although half of the males did not return to the natal site in November. In contrast, 78–90 % of females returned

over the entire 3 month sampling period. It is interesting to note that the average homing percentage of both sexes for 3 months is 83 %, indicating no differences in the total number of homing individuals between male and female. Although male salmonids do not show any territorial behaviour, they maintain high levels of aggressive behaviour to compete for access to females suggesting that early returning males might accrue some benefits in securing females for breeding. The drastic reduction of male homing percentage late in the season may be interpreted in two ways; (1) some males prefer to go to other unsampled breeding sites to find females, (2) some males are prevented from returning to the natal sites by their early death. The occurrence of relatively few non-homing females throughout the sampling period may be related to the following two population-level hypotheses; (1) the conservative protection of these individuals' strain from the disruption of being captured at their natal spawning site, (2) the enhancement of their strain arising from a wild spawning distribution within the lake. The sexual differences in homing behaviour are thought to be reflected by the different steroid hormone profiles between males and females (Sato et al. 1997). In males, the shortening of homing duration coincided with an increase in serum T and 11KT levels. The reduction of homing percentage was associated with decreased serum T levels and increased serum DHP levels. In females, the shortening of homing duration corresponded to an elevation of serum T and DHP levels, and a drop in serum E₂ levels. Further study using our model systems may reveal sexual differences in hormonal control of the homing migration in salmonid fishes with special reference to the early part of the homing migration.

Since GnRH treatment has been reported to be highly effective in inducing GTH release, ovulation and spermiation in teleost fishes (Zohar 1996), we investigated the effect of GnRH analogue (GnRHa) implantation on both homing profiles and serum steroid hormone levels of fish in September (Sato et al. 1997; Kitahashi et al. 1998). The GnRHa implantation was highly efficient in shortening the homing duration, and caused dramatic increases in serum DHP levels in both sexes. An interesting discrepancy was observed between rapidly and slowly returning individual males: rapidly returning males showed higher serum T levels and lower serum DHP levels than slowly returning individual males. To examine the direct action of T and DHP on homing duration, T and DHP were implanted in fish in September in comparison with GnRHa-implantation. GnRHa-implanted fish returned significantly earlier than the control fish regardless of sex. T implantation tended to reduce homing duration in both males and females, but there was no statistical significance. DHP implantation also significantly shortened homing duration in females, but it did not have any significant effect in males. It is quite interesting to note that the direct actions of T and DHP on homing migration are sex dependent. These data suggest that GnRH in the brain stimulates LH release from the pituitary gland, and then LH enhances serum DHP levels in both sexes during the later part of the homing migration in salmonid fishes. GnRH is believed to play a leading role in the homing migration of both sexes (Urano et al. 1999; Makino et al. 2007), but gonadal steroids, especially T and DHP, seem to have sexually different influences on homing migration.

1.3.3 Involvement of sGnRH in Reproductive Migration in Salmon

Involvement of sGnRH in reproductive migration of masu and chum salmon was also examined by means of immunocytochemical technique with an antiserum to sGnRH and in situ hybridization techniques with an oligonucleotide encoding sGnRH precursor. Immunocytochemical analysis demonstrated that a sGnRH immunoreactive bipolar neuron, which might be related to the terminal nerve, was located in the dorsal portion of the olfactory nerve of both species. Immunoelectron microscopy revealed the presence of sGnRH immunoreactive electron-dense granule-like structures, 50 nm in diameter in the olfactory nerve of masu salmon (Kudo et al. 1994). sGnRH immunoreactive neurons, which also showed signals for pro-sGnRH mRNA, were observed in the dorsal portion of the olfactory nerve in chum salmon at the coastal sea, but not in fish at the spawning ground of the natal river (Kudo et al. 1996). These findings suggest that sGnRH may participate in neurotransmission and/or neuromodulation in reproductive migration of salmonids.

1.4 From the Field to the Laboratory: The Specific Effects of Exercise on Sexual Maturation

The interaction between metabolism and sexual maturation in salmonids is currently a focus of attention. Several very recent studies report on the interaction between metabolism and sexual maturation in wild sockeye salmon and in wild chum salmon.

Large-scale microarray studies of wild sockeye salmon along their 1,300 km migration revealed shifts in metabolic processes in the white muscle induced by (combinations of) environmental cues (Miller et al. 2009). Microarray analysis of gill and liver was combined with telemetry in order to correlate gene expression profiles with survival (Evans et al. 2011). Growth hormone and prolactin have shown important changes related to osmoregulation but may also play an important role in final maturation considering increases in prolactin mRNA and growth hormone 1 receptor mRNA near the spawning grounds (Flores et al. 2011). Metabolic delay of maturation may have been the reason that more reproductively advanced sockeye salmon transited through cooler temperatures (Roscoe et al. 2010).

Onuma et al. (2009a, b; 2010a, b, c) showed in wild chum salmon that *fsh β* and *lh β* pituitary mRNA, plasma T, 11KT and E2 levels were increased in maturing chum salmon that were about to start migration. Plasma insulin-like growth factor I (IGF-I) levels were higher in these fishes indicating that IGF-I may act as somatotrophic signal in stimulating the pituitary–gonad axis. Gene expression levels of growth hormone, prolactin and somatolactin in the pituitary were also elevated before the initiation of migration, thereafter expression of growth hormone went down while prolactin remained high until freshwater adaptation. sGnRH in various brain loci has been shown to peak during marine migration. As discussed in this

chapter, we have seen that sGnRH peaks another time in the telencephalon at the branch point to the spawning ground. Elevation of plasma steroid levels, which occurs concomitant with sexual maturation, appears to be antagonistic to hypo-osmoregulatory capacity in salmonids. This has been demonstrated in both Arctic charr (*Salvelinus alpinus* Linnaeus) (Schmitz and Mayer 1993) and Atlantic salmon (McCormick and Naiman 1985; Lundqvist et al. 1989). As such it can be postulated that one of the factors initiating return migration from the sea in these salmonid species is the onset of sexual maturation and the rise of plasma androgen levels (personal comment I. Mayer). This is supported by the fact that in Arctic charr, the larger maturing males return to their native rivers before the smaller immature males. Therefore, it is possible that one of the driving forces initiating the return migration could be the start of sexual maturation, as seawater adaptability (hypo-osmoregulation) starts to be impaired by elevated androgen levels.

However, it is difficult to assess the effects of the metabolic status in field studies because of the influence of other factors that are involved during certain stages of migration (e.g., starvation, osmoregulation, pheromones, temperature, pressure, specific cues of the spawning ground) that cannot be separated from the specific effects of exercise (also Palstra et al. 2010a). In studies on European eel (*Anguilla anguilla* Linnaeus) and rainbow trout, exercise, such as performed by migratory fish species, has been used experimentally to study its effects on the onset of sexual maturation. A large oval shaped swim flume ($6.0 \times 4.0 \times 0.8$ m; 6,000 litres) was designed, constructed, calibrated and used to simulate group-wise migration of eels (Palstra et al. 2008, 2010b) and rainbow trout. Pubertal autumn-spawning seawater-raised female rainbow trouts have swum continuously in the flume at 0.75 body-lengths per second for 10 days (307 km), 20 days (636 km; Palstra et al. 2010a) and 40 days (1176 km). We have applied RNA sequencing of the red and white muscle transcriptome (Palstra and Planas et al. unpublished data; Planas et al. this book) and microarray analysis of the ovary (Palstra et al. 2010a) of rainbow trout to identify hormonal and metabolic actors that are important in switching from the feeding stage contributing to growth and storage of energy reserves to the reproductive migration stage where fish stop eating, start swimming and prepare for reproduction.

1.4.1 The Migration Phenotype Versus the Maturation Phenotype

As in female eels (Palstra et al. 2010b), swimming exercise suppressed oocyte development in trouts, possibly by inhibiting vitellogenin uptake (Palstra et al. 2010a). A swimming-induced ovarian developmental suppression at the start of vitellogenesis during long-term reproductive migration may be a strategy to avoid increased drag resistance due to oocyte growth and to prevent precocious muscle atrophy. What we have shown in rainbow trouts, and in the previous studies on European eels (Palstra and van den Thillart 2010), is that the onset of vitellogenesis is a clear suppression point and an example of phenotypic plasticity during

long-distance reproductive migrations. It thus appears that the physiological processes occurring in the muscle and ovary are conflicting. When there is a need to migrate, energetic processes in the muscle that provide fuel for contraction and for muscle growth are up-regulated and those in the ovary are down-regulated (migration phenotype). When there is a need to start vitellogenesis, the situation in muscle and ovary is reversed (maturation phenotype).

1.4.2 The ‘Swimming On’ and ‘Swimming Off’ Switch: The Mysterious Roles of the Contracting Muscle and Olfaction of Spawning Ground Specific Smell

A long distance navigating migration to the spawning grounds requires fine-tuning between migration and sexual maturation. Two major switches are required: a ‘swimming on’ switch at the start of migration that coincides with the onset of puberty and a ‘swimming off/reproduction on’ switch at the end of migration switching from physical fitness to spawning maturation (Fig. 1.2). The ‘swimming on’ switch thus preludes the migration phenotype and the ‘swimming off/reproduction on’ switch involves switching to the maturation phenotype. Exercise e.g. muscle contraction is assumed to play a major role in the onset of sexual maturation, as are spawning ground specific triggers like olfaction of specific smells during the final stages of homing migration when final maturation is induced.

1.4.2.1 The ‘Swimming On’ Switch

Fishes that migrate long distances to reproduce, especially those that spawn only once in a lifetime like the semelparous salmonid species, have a relatively long feeding stage to grow up to a size where they have the energy stores and they are physically fit to migrate, to mature and to reproduce successfully (Fig. 1.2). At the start of their migration, a metabolic switch is required for the transition from building up reserves to their mobilisation, not only to fuel migration but also to fuel early gonadal maturation. Furthermore, a hormonal switch is required to be sexually mature. The question of how the metabolic status determines the onset of sexual reproduction, or puberty, is still largely unsolved but pivotal in understanding the reproductive physiology of fish. This first step in sexual maturation coinciding with the start of migration (the ‘swimming on’ switch; Fig. 1.2) includes onset of puberty and thus neurohormonal regulation of GTHs production and release.

In a recent study (Palstra and Planas et al. unpublished data), we have obtained important new insights into the role of the muscle during the anorexic reproductive migration of salmonids. We have used the Illumina GA2 sequencing system to provide an in-depth view of the transcriptome of red and white skeletal muscle of exercised and non-exercised rainbow trout (see also Planas et al., this book).

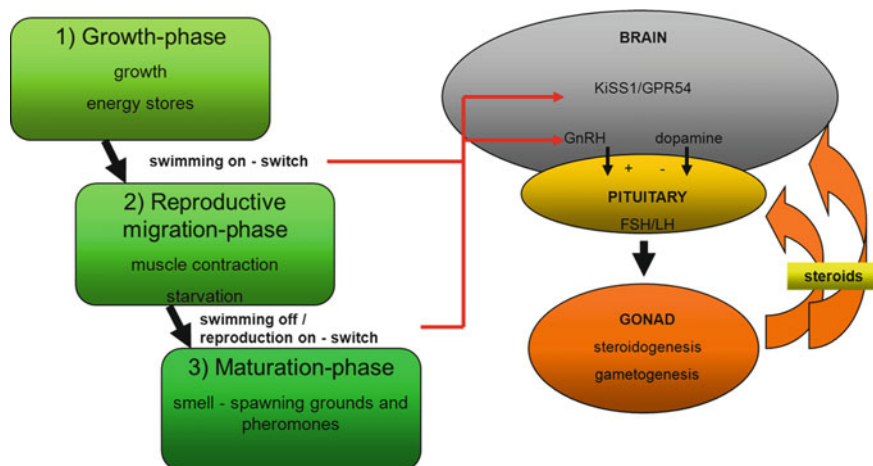


Fig. 1.2 Hypothesized reproductive regulation by GnRH neurohormones and the KiSS1/GPR54 pathway triggered by swimming (muscle contraction) and smell. The life cycle of all fish contains a phase that is characterised by growth and storage of energy. Fish that start migration to their natal spawning grounds (homing) experience a metabolic switch from storing to mobilisation of energy that coincides with an onset of puberty. Since fish switch during a state of starvation and exercise, swimming (e.g. muscle contraction) obviously plays an important role in this switch as well as in suppression of progressing vitellogenesis. A second switch to complete vitellogenesis and induce final maturation is therefore expected when nearing the spawning grounds. At least in salmonid fish smell plays a major role not only in finding the spawning river but also in inducing final maturation. The switches to swimming (by muscle contraction) and reproduction (by smell) may thus represent the triggering of reproductive pathways by GnRH neurohormones, probably through the KiSS1/GPR54 pathway

We have investigated the effects of exercise in red and white muscle and, specifically, focused on muscle building versus wasting processes and on the potential interaction between muscle and the reproductive axis. This information provided more insight to determine whether the rainbow trout muscle matched the migration phenotype or the sexual maturation phenotype after a 40-days long-term exercise of simulated reproductive migration. The results of this study suggested that as long fishes are swimming (and a potential spawning ground trigger is missing), they remain in the migration phenotype. Results showed that both red and white muscle may interact with the reproductive axis through binding of estrogens and Kisspeptin, and muscular production of Vitellogenin and FSH.

1.4.2.2 The 'Swimming Off/Reproduction On' Switch

Since swimming suppresses vitellogenesis, the second major turning point in the sexual maturation of migrant fish, specifically salmonids, may occur when they are near their spawning grounds and energy investments in swimming performance can be turned down low. They now complete their vitellogenesis and undergo final

maturation followed by ovulation and spawning. A spawning ground specific trigger should be involved in switching from swimming to reproduction (the 'swimming off/reproduction on' switch).

Sensory manipulated experiments in lacustrine salmon populations indicated that neither magnetic nor visual cues but, instead, olfactory cues play an important role during the migrational stage in freshwater (this Chapter). Most interestingly, sGnRH levels in the TC and serum testosterone levels in both sexes showed a coincidental peak at the branch point with the river that led to the spawning grounds. These results confirm that sGnRH, besides regulating GTH secretion in the pituitary, plays an olfactory role in chum salmon. This strongly suggests a functional link between olfaction and reproductive regulation with sGnRH playing an important modulating role. In the search for the olfactory discriminating ability of natal stream odours (Yamamoto et al. 2010), glutathione S-transferase class pi enzymes (Kudo et al. 1999), salmon olfactory marker protein (Kudo et al. 2009), salmon olfactory imprinting-related gene (Hino et al. 2007), and olfactory receptors (Morinishi et al. 2007) have been identified as potential factors involved in this process.

1.4.2.3 Perspectives

Muscle contraction and smell should interact on the control levels of reproduction in the migratory fish brain, on the level of neurohormonal GTH releasing hormone (GnRH) and the relatively newly discovered KiSS1/GPR54 system. The environmental triggering of the GnRH system, the functional mechanism and the involvement of the KiSS1/GPR54 system in the migratory fish brain is under current investigation, both in the field as in the laboratory to assess the specific effects of exercise.

1.5 Conclusions

This review describes our recent studies on the progress of sexual maturation during reproductive migration in anadromous and lacustrine Pacific salmon. Using these model fish, two different approaches in connection with homing behaviour in the open water and hormonal control mechanisms of reproductive migration provide several valuable findings on salmon reproductive migration. However, many unknowns still remain such as the imprinting mechanisms during downstream migration, the triggering mechanisms of the shift from feeding migration to spawning migration, the sensory mechanisms of open water orientation and the hormonal control mechanisms for sensory systems. Despite the difficulties to follow their whole life cycle and to wait their sole reproductive timing, comparative behavioural to molecular biological studies using anadromous and lacustrine Pacific salmon will provide a new concept for the physiological mechanisms of imprinting and homing migration in salmon.

The interaction between metabolism and sexual maturation under exercise, as experienced during the salmonid anorexic reproductive migration, has been studied by swimming the facultative migrant rainbow trout in a large swim flume. RNA sequencing of the red and white muscle transcriptome and microarray analysis of the ovary has been performed to identify hormonal and metabolic actors that are important in switching from the growth phase to the migration phenotype ('swimming on' switch). A swimming-induced ovarian developmental suppression at the start of vitellogenesis has been observed during long-term simulated migration. Red and white muscle may interact with the reproductive axis through binding of estrogens and Kisspeptin, and muscular production of Vitellogenin and FSH. Olfaction of spawning ground specific smell is suggested as switch from the migration to the maturation phenotype ('swimming off/reproduction' on switch).

Acknowledgments We would like to express our sincere gratitude to those who have carried out collaborative researches: M. Kaeriyama, H. Tanaka, Y. Naito, S. Urawa, N. Davis, M. Fukuwaka, JBK Leonard, H. Nii, Y. Makiguchi, A. Sato, K. Orito, Y. Konno, K. Miyoshi, National Salmon Center, Hokkaido Fish Hatchery, Hokkaido Farming Fisheries Promotion Corporation, and Chitose Salmon Aquarium for the behavioural study; A. Urano, K. Amano, M. Iwata, S. Adachi, H. Ando, H. Yamada, H. Kudo, M. Zerihun, R.K. Bhandari, M. Fukaya, N. Kozu, T. Kitani, S. Matsumoto, S. Taniyama for the endocrinological study. The present study was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, from the Japan Society for the promotion of Science (JSPS), from the Hokkaido Foundation for the Promotion of Scientific and Industrial Technology, from the Mitsubishi Foundation, from the Mitsui & Co. Ltd, and from the Hokkaido University to HU. AP would specifically like to thank J. Planas, Y. Zohar, G. van den Thillart and F. Sibbing. AP was supported by a Marie Curie Intra-European Fellowship from the European Commission (REPRO-SWIM) with Grant Agreement number 219971 (with J. Planas), a FY2011 JSPS Invitation Fellowship for research in Japan by the Japan Society for the Promotion of Science (No. S-11097 with H. Ueda) and by IMARES.

References

- Amano M, Urano A, Aida K (1997) Distribution and function of gonadotropin-releasing hormone (GnRH) in the teleost brain. *Zool Sci* 14:1–11
- Cooke SJ, Hinch SG, Wikelski M, Andrews RD, Kuchel LJ, Wolcott TG, Butler PJ (2004) Biotelemetry: a mechanistic approach to ecology. *Trend Ecol Evol* 19:334–343
- Dittman AW, Quinn TP (1996) Homing in pacific salmon: mechanisms and ecological basis. *J Exp Biol* 199:83–91
- Evans TG, Hammill E, Kaukinen K, Schulze AD, Patterson DA, English KK, Curtis JM, Miller KM (2011) Transcriptomics of environmental acclimatization and survival in wild adult Pacific sockeye salmon (*Oncorhynchus nerka*) during spawning migration. *Mol Ecol*. doi:10.1111/j.1365-294X.2011.05276.x
- Flores A-M, Shrimpton JM, Patterson DA, Hills JA, Cooke SJ, Yada T, Moriyama S, Hinch SG, Farrell AP (2011) Physiological and molecular endocrine changes in maturing wild sockeye salmon, *Oncorhynchus nerka*, during ocean and river migration. *J Comp Physiol B*. doi:10.1007/s00360-011-0600-4
- Hino H, Iwai T, Yamashita M, Ueda H (2007) Identification of an olfactory imprinting-related gene in the lacustrine sockeye salmon, *Oncorhynchus nerka*. *Aquaculture* 273:200–208

- Kaeriyama M, Ueda H (1998) Life history strategy and migration pattern of juvenile sockeye (*Oncorhynchus nerka*) and chum salmon (*O. keta*) in Japan: a review. NPAFC Bull 1:163–171
- Kitahashi T, Sato A, Alok D, Kaeriyama M, Zohar Y, Yamauchi K, Urano A, Ueda H (1998) Gonadotropin-releasing hormone analog and sex steroids shorten homing duration of sockeye salmon in Lake Shikotsu. Zool Sci 15:767–771
- Kudo H, Ueda H, Kawamura H, Aida K, Yamauchi K (1994) Ultrastructural demonstration of salmon-type gonadotropin-releasing hormone in the olfactory system of masu salmon (*Oncorhynchus masou*). Neurosci Lett 166:187–190
- Kudo H, Hyodo S, Ueda H, Hiroi O, Aida K, Urano A, Yamauchi K (1996) Cytophysiology of gonadotropin-releasing-hormone neurons in chum salmon (*Oncorhynchus keta*) forebrain before and after upstream migration. Cell Tissue Res 284:261–267
- Kudo H, Ueda H, Mochida K, Adachi S, Hara A, Nagasawa H, Doi Y, Fujimoto S and Yamauchi K (1999) Salmonid olfactory system-specific protein (N24) exhibits glutathione S-transferase class pi-like structure. J Neurochem 72:1344–1352
- Kudo H, Doi Y, Ueda H and Kaeriyama M (2009) Molecular characterization and histochemical demonstration of salmon olfactory marker protein in the olfactory epithelium of lacustrine sockeye salmon (*Oncorhynchus nerka*). Comp Biochem Physiol 154A:142–150
- Lundqvist H, Borg B, Berglund I (1989) Androgens impair seawater adaptability in smolting Baltic salmon (*Salmo salar*). Can J Zool 67:1733–1736
- Makino K, Onuma T, Kitahashi T, Ando H, Ban M, Urano A (2007) Expression of hormone genes and osmoregulation in homing chum salmon: a minireview. Gen Comp Endocrinol 152:304–309
- McCormick SD, Naiman RJ (1985) Hypoosmoregulation in an anadromous teleost: influence of sex and maturation. J Exp Zool 234:193–198
- Miller KM, Schulze AD, Ginther N, Li S, Patterson DA, Farrell AP, Hinch SG (2009) Salmon spawning migration: metabolic shifts and environmental triggers. Comp Biochem Physiol 4D:75–89
- Morinishi F, Shiga T, Suzuki N, Ueda H (2007) Cloning and characterization of an odorant receptor in five Pacific salmon. Comp Biochem Physiol 148B:329–336
- Nagahama Y (1999) Gonadal steroid hormones: Major regulators of gonadal differentiation and gametogenesis in fish. In: Norberg B, Kjesbu OS Taranger GL, Andersson E, Stefansson SO (eds) Proceedings of the 6th international symposium on the reproductive physiology of fish, Bergen, pp 211–222
- Onuma TA, Sato S, Katsumata H, Makino K, Hu WW, Jodo A, Davis ND, Dickey JT, Ban M, Ando H, Fukuwaka M, Azumaya T, Swanson P, Urano A (2009a) Activity of the pituitary-gonadal axis is increased prior to the onset of spawning migration of chum salmon. J Exp Biol 212:56–70
- Onuma TA, Makino K, Ban M, Ando H, Fukuwaka M, Azumaya T, Swanson P, Urano A (2009b) Elevation of the plasma level of insulin-like growth factor-I with reproductive maturation prior to initiation of spawning migration of chum salmon. Ann NY Acad Sci 1163:497–500
- Onuma TA, Makino K, Katsumata H, Beckman BR, Ban M, Ando H, Fukuwaka M, Azumaya T, Swanson P, Urano A (2010a) Changes in the plasma levels of insulin-like growth factor-I from the onset of spawning migration through upstream migration in chum salmon. Gen Comp Endocrinol 165:237–243
- Onuma TA, Ban M, Makino K, Katsumata H, Hu WW, Ando H, Fukuwaka M, Azumaya T, Urano A (2010b) Changes in gene expression for GH/PRL/SL family hormones in the pituitaries of homing chum salmon during ocean migration through upstream migration. Gen Comp Endocrinol 166:537–548
- Onuma TA, Makino K, Ando H, Ban M, Fukuwaka M, Azumaya T, Urano A (2010c) Expression of GnRH genes is elevated in discrete brain loci of chum salmon before initiation of homing behavior and during spawning migration. Gen Comp Endocrinol 168:356–368
- Palstra AP, van den Thillart GEEJM (2010) Swimming physiology of eels: energetic costs and effects on sexual maturation and reproduction. Fish Physiol Biochem 36:297–322

- Palstra AP, Schnabel D, Nieveen MC, Spaik HP, van den Thillart GEEJM (2008) Male silver eels mature by swimming. *BMC Physiol* 8:14
- Palstra AP, Crespo D, van den Thillart GEEJM, Planas JV (2010a) Saving energy to fuel exercise: swimming suppresses oocyte development and down-regulates ovarian transcriptional response of rainbow trout *Oncorhynchus mykiss*. *Am J Physiol Reg I* 299:R486–R499
- Palstra AP, Schnabel D, Nieveen MC, Spaik HP, van den Thillart G (2010b) Swimming suppresses hepatic vitellogenesis in European silver eel as shown by quantitative RT-PCR of the estrogen receptor 1, vitellogenin1 and vitellogenin2 in the liver. *Reprod Biol Endocrinol* 8:27
- Quinn TP (2005) The behaviour and ecology of pacific salmon and trout. University of Washington Press, Seattle
- Roscoe DW, Hinch SG, Cooke SJ, Patterson DA (2010) Behaviour and thermal experience of adult sockeye salmon migrating through stratified lakes near spawning grounds: the roles of reproductive and energetic states. *Ecol Freshw Fish* 19:51–62
- Sato A, Ueda H, Fukaya F, Kaeriyama M, Zohar Y, Urano A, Yamauchi K (1997) Sexual differences in homing profiles and shortening of homing duration by gonadotropin-releasing hormone analog implantation in lacustrine sockeye salmon (*Oncorhynchus nerka*) in Lake Shikotsu. *Zool Sci* 14:1009–1014
- Schmitz M, Mayer I (1993) Effects of androgens on seawater adaptation in Arctic charr, *Salvelinus alpinus*. *Fish Physiol Biochem* 12:11–20
- Tanaka H, Naito Y, Davis ND, Urawa S, Ueda H, Fukuwaka M (2005) Behavioural thermoregulation of chum salmon during homing migration in coastal waters. *Mar Ecol Prog Ser* 291:307–312
- Ueda H (1999) Artificial control of salmon homing migration and its application to salmon propagation. *Bull Tohoku Nat Fish Res Inst* 62:39–133
- Ueda H (2004) Recent biotelemetry research on lacustrine salmon homing migration. *Mem Natl Inst Polar Res Spec Issue* 58:80–88
- Ueda H (2011) Physiological mechanisms of homing migration in Pacific salmon from behavioral to molecular biological approaches. *Gen Comp Endocrinol*. doi:10.1016/j.ygen.2010.02.003
- Ueda H, Yamauchi K (1995) Biochemistry of fish migration. In: Hochachka PW, Mommsen TP (eds) *Biochemistry and molecular biology of fishes Vol 5*, Elsevier, Amsterdam, pp 265–279
- Ueda H, Hiroi O, Hara A, Yamauchi K, Nagahama Y (1984) Changes in serum concentrations of steroid hormone, thyroxine, and vitellogenin during spawning migration of chum salmon, *Oncorhynchus keta*. *Gen Comp Endocrinol* 53:203–211
- Ueda H, Kaeriyama M, Mukasa K, Urano A, Kudo H, Shoji T, Tokumitsu Y, Yamauchi K, Kurihara K (1998) Lacustrine sockeye salmon return straight to their natal area from open water using both visual and olfactory cues. *Chem Senses* 23:207–212
- Ueda H, Leonard JBK, Naito Y (2000) Physiological biotelemetry research on the homing migration of salmonid fishes. In: Moore A, Russell I (eds) *Advances in fish telemetry*. Crown Copyright, Lowestoft, pp 89–97
- Urano A, Ando H, Ueda H (1999) Molecular neuroendocrine basis of spawning migration in salmon. In: Kwon HB, Joss JMP, Ishii S (eds) *Recent progress in molecular and comparative endocrinology*. Hormone Research Center, Kwangju, pp 46–56
- Walker MM, Diebel CE, Haugh CV, Pankhurst PM, Montgomery JC, Green CR (1997) Structure and function of the vertebrate magnetic sense. *Nature* 390:371–376
- Yamada H, Amano M, Okuzawa K, Chiba H, Iwata M (2002) Maturation changes in brain contents of salmon GnRH in rainbow trout as measured by a newly developed time-resolved fluoroimmunoassay. *Gen Comp Endocrinol* 126:136–143
- Yamamoto Y, Hino H, Ueda H (2010) Olfactory imprinting of amino acids in lacustrine sockeye salmon. *PLoS ONE*. doi:10.1371/journal.pone.0008633
- Zohar Y (1996) New approaches for the manipulation of ovulation and spawning in farmed fish. *Bull Natl Res Inst Aquacult Suppl* 2:43–48

Swimming Physiology of Fish
Towards Using Exercise to Farm a Fit Fish in
Sustainable Aquaculture
Palstra, A.P.; Planas, J.V. (Eds.)
2013, X, 430 p., Hardcover
ISBN: 978-3-642-31048-5