



## Physiological mechanisms of imprinting and homing migration in Pacific salmon *Oncorhynchus* spp.

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After several years of feeding at sea, salmonids have an amazing ability to migrate long distances from the open ocean to their natal stream to spawn. Three different research approaches from behavioural to molecular biological studies have been used to elucidate the physiological mechanisms underpinning salmonid imprinting and homing migration. The study was based on four anadromous Pacific salmon *Oncorhynchus* spp., pink salmon *Oncorhynchus gorbuscha*, chum salmon *Oncorhynchus keta*, sockeye salmon *Oncorhynchus nerka* and masu salmon *Oncorhynchus masou*, migrating from the North Pacific Ocean to the coast of Hokkaido, Japan, as well as lacustrine *O. nerka* and *O. masou* in Lake Toya, Hokkaido, where the lake serves as the model oceanic system. Behavioural studies using biotelemetry techniques showed swimming profiles from the Bering Sea to the coast of Hokkaido in *O. keta* as well as homing behaviours of lacustrine *O. nerka* and *O. masou* in Lake Toya. Endocrinological studies on hormone profiles in the brain–pituitary–gonad axis of *O. keta*, and lacustrine *O. nerka* identified the hormonal changes during homing migration. Neurophysiological studies revealed crucial roles of olfactory functions on imprinting and homing during downstream and upstream migration, respectively. These findings are discussed in relation to the physiological mechanisms of imprinting and homing migration in anadromous and lacustrine salmonids.

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### INTRODUCTION

To maximize reproductive success, migratory fishes move, either alone or in schools, from their birth place to their growth habitats and then usually move back to their original birth place to spawn. These migrations allow fishes not only to exploit different food availabilities and to adapt to environmental changes but also to regulate population density and widen their distribution. One of the most interesting aspects of fish migration is imprinting and homing migration to the place of natal origin, but many of the physiological mechanisms of imprinting and homing migration in fishes remain a mystery. In this review, physiological mechanisms of fish migration are described with special reference to salmonid imprinting and homing migration by adding evolutionary points of view.

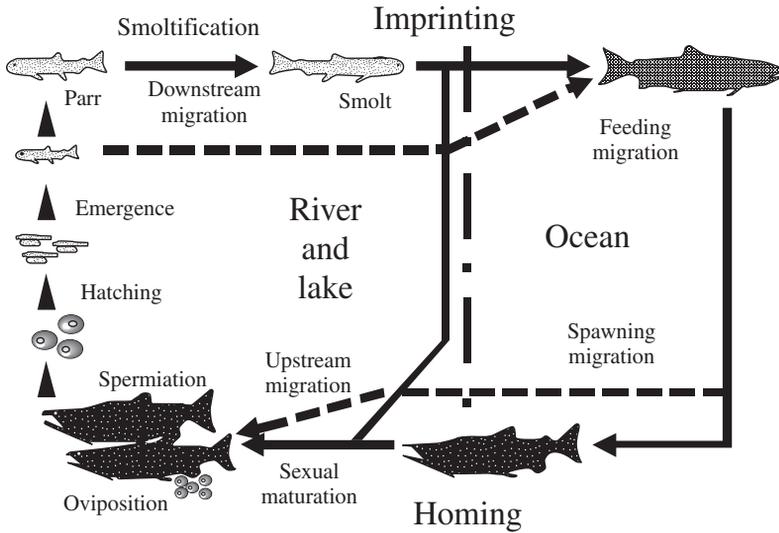


FIG. 1. Two different types of life history of Pacific *Oncorhynchus* species in Japan: - - -, *Oncorhynchus keta* and *Oncorhynchus gorbuscha* and —, *Oncorhynchus nerka* and *Oncorhynchus masou*.

### LIFE CYCLE OF PACIFIC SALMON *ONCORHYNCHUS* SPP. IN JAPAN

There are four Pacific salmon *Oncorhynchus* species in Japan: pink salmon *O. gorbuscha* (Walbaum 1792), chum salmon *Oncorhynchus keta* (Walbaum 1792), sockeye salmon *Oncorhynchus nerka* (Walbaum 1792) and masu salmon *Oncorhynchus masou* (Brevoort 1856). The life cycles of the first two species are quite different from the last two species (Fig. 1). All juvenile fry of *O. gorbuscha* and *O. keta* make their downstream migration a few months after emergence, and adult fishes make their upstream migration a few weeks before final gonadal maturation. Conversely, smolts of *O. nerka* and *O. masou* stay for a year and a half in streams or lakes, before their downstream migration and becoming adapted to seawater. The adults perform their upstream migration several months before spawning. There are also land-locked forms (lacustrine) of both *O. nerka* and *O. masou* (Groot & Margolis, 1991).

Of the four *Oncorhynchus* species, *O. gorbuscha* are considered phylogenetically, (using retropositional genome analyses) to be the most advanced while *O. masou* are considered to be the more primitive species (Murata *et al.*, 1996). Analyses of the relationship between the oceanic distribution and the population size among the four species reveal that *O. gorbuscha* are distributed most widely and have the largest population while *O. masou* appear to have the most restricted distribution and have the smallest population (Kaeriyama & Ueda, 1998). Although the homing accuracy of these fishes has not been examined in detail, it is believed that *O. masou* come back to their natal stream with the highest percentage and that *O. gorbuscha* stray off into the non-natal stream. If salmonids always show a highly accurate homing to their natal stream, there would be little chance to widen their distribution area or increase their population size and there is the dangerous possibility that this may reduce their genetic diversity. The relationship between salmonid evolution and homing accuracy is one of the most interesting problems from a biological evolution point of view.

## SALMONID HOMING MIGRATION

Since the olfactory hypothesis for salmonid homing was proposed by Hasler & Scholz (1983), it has become widely accepted that some specific odourant factors of the natal stream are imprinted to the olfactory system of juvenile salmonids during their downstream migration and that adult salmonids recognize these factors from their natal stream during their homing migration. This olfactory capability is believed only to be exerted close to the coast, within a short distance of the coast of the natal stream and therefore it must be impossible for salmonids to use only this capability to direct their long distance migration from the open-ocean feeding area to their natal area. For open-water orientation and navigation of salmonids, mechanisms such as a map-and-compass system and rheotaxis in relation to oceanic currents have been discussed (Quinn & Groot, 1984; Hansen *et al.*, 1993; Dittman & Quinn, 1996; Quinn, 2005). Moreover, in order to calculate the time needed to travel from the feeding habitat to the natal stream as well as from the stream mouth to the spawning area, salmonids must have an accurate biological clock, but there have been few references to the role of a biological clock in salmonid migration. It is still therefore unclear which sensory systems play leading roles in open-water orientation or how the olfactory system discriminates various stream odours. Both *O. keta* that migrate from the Bering Sea to the coast of Hokkaido, and lacustrine *O. nerka* and *O. masou* in Lakes Toya (42° 34' N; 140° 50' E) and Shikotsu (42° 45' N; 141° 20' E) in Hokkaido where the lakes serve as a model ocean, offer good systems for studying the physiological mechanisms of imprinting and homing abilities in salmonids. Three different physiological approaches have been used; behavioural studies using biotelemetry techniques, endocrinological studies involving the brain–pituitary–gonad axis and the sensory neurophysiological studies on olfactory functions.

## BEHAVIOURAL RESEARCH

The recent rapid development in biotelemetry techniques, such as ultrasonic and radio telemetry, data logging and pop-up satellite telemetry, make it possible to investigate wild animal behaviour both on land and in the ocean. The continuous observation of underwater behaviour of salmonids in open water that was impossible to monitor by previous techniques can now be analysed in detail (Cooke *et al.*, 2004; Ueda, 2004).

*Oncorhynchus keta* were caught by longline in June 2000 in the central Bering Sea in a healthy condition and were estimated to be of 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 at the dorsal musculature of the fish anterior to the dorsal fin. Twenty-seven *O. keta* with these data loggers were released and one data logger was retrieved in September 2000 from a set net on the east coast of Hokkaido, Japan. This first record of swimming profiles of homing *O. keta* in the oceanic phase, provided data for 67 days over a straight distance of 2750 km and revealed that mean  $\pm$  s.d. swimming speed, depth and temperature were  $62 \pm 12$  cm s<sup>-1</sup>,  $10.4 \pm 14.7$  m and  $9.2 \pm 0.2^\circ$  C (Tanaka *et al.*, 2005). Both swimming speed and depth had two peaks around the time of sunrise and sunset with a further small peak around midnight. The fish showed sequential up-and-down movement near the thermocline during twilight and daytime. These

diurnal patterns suggest that homing *O. keta* allocated its time to foraging and that strategies were different between daytime and night-time. These results indicate that homing *O. keta* have an ability to navigate in their homeward direction and transport by water currents may help the successful migration. During migration, salmonids must recognize exact location (map) and compass direction (orientation) and must have a biological clock for accurate homing in open water.

For sea-run anadromous populations, however, it is difficult to carry out controlled and manipulated physiological experiments as fishes move from the sea, in their prematuration phase, to their natal stream, where they become mature. In contrast, lacustrine populations offer a good model system for studying the homing mechanisms of salmonids from open water to their natal area where they spawn. Lake Toya (surface area 71 km<sup>2</sup>, average and maximum depth 116 and 179 m) is a large caldera lake in Hokkaido, Japan. The homing behaviours of mature lacustrine *O. nerka*, whose sensory cues were impaired, were tracked from the centre of the lake to the natal area using an ultrasonic tracking system (Ueda *et al.*, 1998). Both a mature male *O. nerka* equipped with a brass ring (control) and a similar mature male *O. nerka* whose magnetic cues were interfered with by means of a magnetic ring returned straight to the natal area after 1 h of random movement. A mature male *O. nerka* whose visual and magnetic cues were both blocked moved in a direction opposite to the natal area, but was rediscovered in the natal area on the following evening, suggesting the possible involvement of olfactory cues in finding the natal area. A blinded male was also moved to the shore of Naka-Toya far from the natal area in the evening, where it stayed for a few days. These data suggest that visual cues are critical to the straight homing of *O. nerka*, while magnetic cues do not appear to be necessary for successful return to the natal area. Magnetoreceptor cells, however, have been identified in the nose of rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Walker *et al.*, 1997). Further study is needed to investigate the involvement of magnetic cues during oceanic homing migration in salmonids.

The homing behaviours of mature lacustrine *O. masou* were also tracked in Lake Toya (Ueda *et al.*, 2000). A mature control male moved constantly along the coast and stopped its movement at the mouth of stream. A blinded mature female was released and moved randomly away from the coast. A mature male *O. masou* whose olfactory cue was blocked moved randomly along the coast and then tended to move away from the coast. It is interesting to compare the straight movements of *O. nerka* with the coastal movement behaviours of *O. masou*. These two species show large differences in oceanic distribution. *Oncorhynchus nerka* are distributed widely in the North Pacific Ocean, while *O. masou* have a more restricted distribution in the western North Pacific Ocean (Kaeriyama & Ueda, 1998). These data suggest some ecological aspects of successful homing migration of salmonids where the more narrowly distributed *O. masou* only need coastal recognition ability, but the more widely distributed *O. nerka* must obtain open-water cues for orientation. Moreover, lacustrine *O. masou* in Lake Toya showed clear diurnal movement when they encountered the river mouth for the first time at the beginning of spawning season. They swam at *c.* thermocline depth in the daytime and stayed at the water surface during the night. This diurnal movement disappeared gradually towards the peak of the spawning season, and they carried out upstream migration to the spawning ground in rivers (unpubl. data). These behavioural changes in *O. masou* during the spawning season suggested that they are able to calculate the day length using a biological clock.

## ENDOCRINOLOGICAL RESEARCH

The homing migration in salmonids is closely related to gonadal maturation, and this is regulated mainly by the brain–pituitary–gonad (BPG) axis. There are two molecular types of gonadotropin-releasing hormone (GnRH), salmon GnRH (sGnRH) and chicken II GnRH (cGnRH-II) in salmonid brains (Amano *et al.*, 1997). In particular, sGnRH in the olfactory system, the terminal nerve and the preoptic area are considered to play important roles in homing migration of salmonids. Subsequently, 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; oestradiol-17 $\beta$  (E<sub>2</sub>) 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, 1997). Hormone profiles have been investigated in the BPG axis of salmonids during homing migration and gonadal maturation (Ueda & Yamauchi, 1995; Ueda, 1999, 2011; Urano *et al.*, 1999; Makino *et al.*, 2007).

The hormone profiles in the BPG axis of *O. keta* migrating from the Bering Sea to the spawning ground in the Chitose River, Hokkaido, Japan (43° 07' N; 141° 34' E), were measured using specific time-resolved fluoroimmunoassay (TR-FIA) systems developed by 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 stream mouth of the Ishikari River (43° 16' N; 141° 22' E) 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 around the coastal sea in females and around the stream mouth of the Ishikari River in males. In contrast, FSH levels did not show any clear correlations 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 pre-spawning 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 (Ueda, 2011).

sGnRH immunoreactive neurons, which also showed signals for pro-sGnRH messenger (m)RNA, were observed in the dorsal portion of the olfactory nerve in *O. keta* in the coastal sea, but not in fish at the spawning ground in the natal river (Kudo *et al.*, 1996a). Changes in the levels of GTH subunit mRNAs in the pituitary of pre-spawning *O. keta* reported that the levels of GTH  $\alpha$ 2 and LH $\beta$  increased from seawater to fresh water, but these of FSH $\beta$  showed no significant changes (Kitahashi *et al.*, 1998a). Serum steroid hormone levels showed similar profiles as previous observations (Ueda *et al.*, 1984; Ueda, 1999); E<sub>2</sub> 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 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 that sGnRH plays a role in GTH secretion in the pituitary of *O. keta*, and sGnRH and cGnRH-II might be involved in brain

region-dependent roles on gonadal maturation and homing migration in salmonids. In addition, year-to-year differences in plasma levels of steroid hormones in pre-spawning *O. keta* were also studied in relation to sea surface temperature (SST) of the coastal sea, and showed year-to-year differences in plasma levels of steroid hormones and gonadal maturity, some of which may be influenced by year-to-year variation of SST (Onuma *et al.*, 2003).

In Lake Shikotsu (surface area 78 km<sup>2</sup>, average and maximum depth 265 and 363 m, respectively), adult *O. nerka* 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. Fish were sampled again at recapture to characterize 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 of both sexes for 3 months is 83%, indicating no differences in the total number of homing individuals between males and females. Although only old references were available about studying the sex ratio of *O. keta* on the spawning ground, Bakkala (1970) reported that males predominated early and females late in the spawning run. Although male salmonids do not show any territorial behaviours, they maintain high levels of aggressive behaviour to compete for access to females (Jones, 1959) suggesting that early returning males might accrue some benefits in securing females for breeding.

The differences between the sexes 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 E<sub>2</sub> levels. Since GnRH treatment has been reported to be highly effective in inducing GTH release, ovulation and spermiation in teleosts (Zohar, 1996), the effect of GnRH analogue (GnRH<sub>a</sub>) implantation on both homing profiles and serum steroid hormone levels of fish in September was investigated (Sato *et al.*, 1997; Kitahashi *et al.*, 1998b). The GnRH<sub>a</sub> 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 GnRH<sub>a</sub> implantation (Kitahashi *et al.*, 1998b). GnRH<sub>a</sub>-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. These steroid hormone implantations did not affect serum T and DHP levels. It is interesting to note that the direct actions of T and DHP on homing migration are sex dependent.

The peak of plasma T levels in land-locked *O. nerka* of both sexes was observed at the time when they gathered at the mouth of their natal stream in Lake Chuzenji, Honshu, Japan (36° 44' N; 139° 28' E) (Ikuta, 1996). Androgens are well-known to be involved in stimulating aggressive behaviour in teleosts (Villars, 1983), and serum T and 11KT are the two major androgens that influence spawning behaviours, downstream and upstream migration and the social dominance hierarchy (Kindler *et al.*, 1989; Cardwell & Liley, 1991; Pankhurst & Barnett, 1993; Brantly *et al.*, 1993; Cardwell *et al.*, 1996; Munakata *et al.*, 2001a, b). Although DHP is known to be a maturation-inducing steroid in salmonids (Nagahama & Adachi, 1985), its function to the central nervous system has not yet been clarified. The functional roles of T and DHP on the salmonid homing migration should be further investigated with special attention to their action on the central nervous system.

GnRHa implantation was also highly effective in accelerating gonadal maturation in anadromous, maturing *O. nerka* of both sexes. Expression of GTH subunit genes in the pituitary gland was examined and revealed that the levels of GTH $\alpha$  and LH $\beta$  mRNAs in GnRHa-implanted fish were higher than those in control fish, but the levels of FSH $\beta$  mRNA showed no change (Kitahashi *et al.*, 1998c). Implantation of GnRHa caused a significant elevation of serum DHP levels in both sexes, but had no effect on levels of T and 11KT in males or E<sub>2</sub> and T in females (Fukaya *et al.*, 1998).

These data suggest that sGnRH in the brain stimulates LH release from the pituitary gland and then LH enhances serum DHP levels in both sexes during the latter part of the homing migration in salmonids. GnRH is believed to play a leading role in the homing migration of both sexes, but gonadal steroids, especially T and DHP, seem to have sexually different influences on homing migration. Further study using the model systems may reveal sexual differences in hormonal control of the homing migration in salmonids with special reference to the early part of the homing migration.

## OLFACTORY RESEARCH

Since the olfactory hypotheses for salmonid imprinting and homing to their natal stream was proposed by Hasler & Wisby, (1951) and Wisby & Hasler (1954), mechanisms of olfactory imprinting and homing abilities in salmonids have been intensively studied (Hasler & Scholz, 1983; Døving, 1989; Stabell, 1992; Bertmar, 1997; Nevitt & Dittman, 1998; Ueda *et al.*, 2007; Hino *et al.*, 2009; Ueda, 2011). The pheromone hypothesis proposed by Nordeng (1971, 1977) using Arctic charr *Salvelinus alpinus* (L. 1758) and Atlantic salmon *Salmo salar* L. 1758 suggested that juvenile salmonids in a stream released population-specific odours that guided homing adults. Several studies have also suggested that juvenile salmonids produce population-specific odours or pheromones (Groot *et al.*, 1986; Quinn & Tolson, 1986; Courtenay *et al.*, 1997). It has also been demonstrated that sex steroids and prostaglandins that have effects on the olfactory epithelium of salmonids may be acting as sexual pheromones (Moore & Scott, 1992; Moore & Warning, 1996). Recently, L-kynurenine, an amino acid, was identified as a sex pheromone in the urine of ovulated female *O. masou* (Yambe *et al.*, 2006). There were no juveniles of *O. keta* or *O. gorbuscha*, however, present at the time that the adults return. Nonetheless, it is now widely accepted that

some specific odours in the natal stream are important for olfactory imprinting and homing in salmonids.

The olfactory discriminatory ability of lacustrine *O. nerka* and *O. masou*, which were reared in the culture pond at Toya Lake Station, was examined by recording the integrated olfactory nerve response (Sato *et al.*, 2000). The olfactory organs of both species elicited different responses to various fresh waters, regardless of sex or gonadal maturity. The source and effluent water from the culture pond evoked the minimum and maximum responses, respectively. These odours may modify the source water in such a way as to make the culture pond water more detectable to the olfactory system. In cross-adaptation experiments, the stream waters abolished the secondary response to the lake water, but the lake water did not abolish the secondary response to the stream waters. This phenomenon is quite reasonable because the salmonids migrate from the lake to the stream. The minimum concentration (threshold) required to induce the olfactory nerve response to the culture pond water after adaptation to the lake water was between 0.1 and 1.0%. This threshold level suggests that the olfactory discriminatory ability of salmonids during homing migration must function within a limited distance from the natal stream.

Several attempts to identify the natal-stream odour were made based on the olfactory bulbar response and suggested that the natal-stream odours were non-volatile (Fagerlund *et al.*, 1963; Cooper *et al.*, 1974; Bodznick, 1978). Spectral analysis of the olfactory bulbar response suggested that the natal-stream odour was absorbed on activated carbon and ion-exchange resin, insoluble in petroleum-ether, dialyzable, non-volatile and heat-stable (Ueda, 1985). Unlike olfactory organs of terrestrial animals, fish olfactory organs respond only to a limited number of chemical species dissolved in water. Chemicals that elicit the response from the olfactory organs of salmonids are amino acids, steroids, bile acids, nucleotides and prostaglandins (Hara, 1994).

The composition of dissolved free amino acids (DFAA), inorganic cations and bile acids in waters from three streams which flow into Lake Toya was examined (Shoji *et al.*, 2000). Application of mixtures of inorganic cations or bile acids, combined based on their compositions in stream waters, to the olfactory epithelium induced only very small responses. On the other hand, application of mixtures of DFAA induced large responses. The response to artificial stream water based on the composition of DFAA and salts closely resembled the response to the corresponding natural stream water. Cross-adaptation experiments with three combinations of natural and artificial stream waters were carried out. The response pattern for each combination of artificial stream water closely resembled that of the corresponding combination of natural stream water. Accordingly, the DFAA compositions in the natal-stream water are probably natal-stream odours. Changes in the DFAA compositions in stream water are attributed mainly to complicated biological processes in the watershed ecosystem. There are many possible factors affecting the DFAA compositions both within and beyond the stream environment, such as soils, vegetation, litter, pollen, dew and various microbial activities (Thomas, 1997). Among these factors, the roles of complex microbial communities called biofilms have been intensively investigated (Costerton *et al.* 1994; Nosyk *et al.* 2008). A biofilm consists of various microorganisms and is embedded into a matrix of extracellular polymeric substances. The origin of DFAA in stream water was investigated focusing on biofilms in the stream bed by means of incubation experiments in the laboratory. Stones were placed in

the Toyohira Stream, Hokkaido (42° 53' N; 141° 9' E), for 3 months, allowing formation of biofilms and then incubated for 24 h in the laboratory at stream-water temperature. After incubation, the composition and concentrations of DFAA in the incubation solution were measured by a high-performance liquid chromatography (HPLC). The DFAA concentration increased greatly in the biofilm incubation solution of the treatment group, but the DFAA composition (mole %) showed little change after 24 h incubation, which was similar to stream water. These results suggest that biofilms are a major source of DFAA in stream water (Ishizawa *et al.*, 2010).

Behaviour experiments were carried out to test attractant effects on upstream selective movement among the four *Oncorhynchus* species using artificial natal-stream water (ANW) prepared to the same composition and concentration of DFAA in their natural natal stream in a two-choice test tank (Y-maze) consisted of two water inlet arms and one pool. Either ANW or natural lake water (NLW) was added to the water inlet of either left or right arms and the fish movement monitored to determine the number of fish that moved to each arm. In a choice of ANW and NLW, *O. gorbuscha* showed the highest percentage of upstream movement among the four *Oncorhynchus* species, but showed the lowest percentage of selectivity in the arm running ANW (Ueda, 2011). These results indicated that ANW had different attractant effects on selective upstream movement among the four *Oncorhynchus* species. *Oncorhynchus gorbuscha* showed the highest upstream movement but the lowest selectivity to the artificial natal-stream water. It is interesting to note that the evolutionary relationship between the olfactory discriminating ability and the homing accuracy among the four *Oncorhynchus* species. If salmonids conduct very accurate homing migrations to their natal stream, there would be little chance to expand their distribution area which would in turn affect population size and genetic diversity. Thus, *O. gorbuscha* may have evolved the capacity to select non-natal-stream odours during homing migration.

Further behavioural experiments with *O. keta* captured in the River Osaru (RO; 42° 28' N; 140° 50' E), Hokkaido, were also conducted in the Y-maze using various combinations of control water (NLW) and three artificial stream waters prepared by using the same composition and concentration of DFAA found in natural stream waters: (1) artificial RO water (ARO), (2) ARO without L-glutamic acid, the major amino acid in RO water (ARO-E) and (3) another artificial water (ALS) had much higher amino-acid concentrations than RO (Yamamoto & Ueda, 2009). In behavioural tests, the fish did not discriminate between ARO and ARO-E, but displayed significant selection of ARO or ARO-E over NLW and ARO over ALS. Electrophysiological cross-adaptation experiments indicated that mature male *O. keta* have the olfactory capability to distinguish between ARO and ARO-E. These results suggest that migratory male *O. keta* respond to DFAA mixtures in their natal-stream water and appear not to be affected by single amino acids.

By using artificial odours,  $\beta$ -phenylethyl alcohol (PEA) or morpholine, coho salmon *Oncorhynchus kisutch* (Walbaum 1792) that had been imprinted with these odours during parr–smolt transformation (PST) were lured into unfamiliar streams scented with these odours during homing migration a few years later (Cooper *et al.*, 1976; Scholz *et al.*, 1976). The olfactory receptor cells of *O. kisutch* that had been imprinted with PEA had a higher sensitivity to PEA as compared with non-imprinted fish (Nevitt *et al.*, 1994), and only fish that were exposed to PEA or natural stream odours during PST formed an imprinted memory (Dittman *et al.*,

1996). Using electrophysiological, behavioural and molecular biological experiments, it was revealed that 1-year-old lacustrine *O. nerka* could be imprinted by a single amino acid, Pro or Glu around PST, and that maturing and matured fish that were exposed to these test waters before and during PST 2 years previously had the ability to select these test waters (Yamamoto *et al.*, 2010).

Recently, blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) has been applied to investigate the odour information processing of natal stream in the brain of lacustrine *O. nerka*, and it was found that strong responses to natal-stream odours were mainly observed in the lateral area of dorsal telencephalon (DI), which are homologous to the medial pallium (hippocampus) in terrestrial vertebrates (Bandoh *et al.*, 2011). Olfactory memory plays a key role in imprinting and recalling natal-stream odour information in salmonids. In the formation of memory, the possible role of long-term potentiation (LTP) has been studied with a focus on *N*-methyl-D-aspartate (NMDA) receptors, which induce LTP (Martin *et al.*, 2000). Further molecular biological studies on functions of NMDA receptor gene in the DI area should be carried out to clarify the olfactory memory processing in salmon.

Using sodium dodecyl sulphate–polyacrylamide gel electrophoresis, an olfactory system-specific protein of 24 kDa (N24) was identified in lacustrine *O. nerka* by electrophoretic comparison of proteins restricted to the olfactory system with those found in other parts of the brain (Shimizu *et al.*, 1993). In various species of teleosts, N24 immunoreactivity was found in the olfactory system of species migrating between the sea and freshwater streams, such as Japanese eel *Anguilla japonica* Temminck & Schlegel 1846, but not in non-migratory species, such as carp *Cyprinus carpio* L. 1758 (Ueda *et al.*, 1994). Interestingly, N24 immunoreactivity was also observed in the testicular germ cells, spermatids and spermatozoa, suggesting its involvement in sperm chemotaxis (Ueda *et al.*, 1993). Immunocytochemical and immunoelectron-microscopic observations revealed that N24-positive immunoreactivity occurred in ciliated and microvillar olfactory receptor cells and the glomerular layer near the mitral cells in the olfactory bulb (Kudo *et al.*, 1996b; Yanagi *et al.*, 2004). Complementary (c)DNA encoding N24 was isolated and sequenced, and this cDNA contained a coding region encoding 216 amino acid residues. Protein and nucleotide sequencing demonstrated the existence of a remarkable homology between N24 and glutathione *s*-transferase class pi enzymes (Kudo *et al.*, 1999). Northern analysis showed that N24 mRNA with a length of 950 bases was expressed in lacustrine *O. nerka* olfactory epithelium. The functional roles of N24 during salmonid homing migration are still unclear, but N24 is a useful molecular marker for studying olfactory functions in salmonids.

Salmonid olfactory imprinting-related gene (*soig*) from the olfactory system of lacustrine *O. nerka* has been identified by subtractive hybridization technique of cDNA representational difference analysis (cDNA-RDA) using fish at PST as a tester and fish at the feeding migration term as a driver (Hino *et al.*, 2007). *soig* mRNA was shown to be expressed in olfactory receptor cells and basal cells of the olfactory epithelium. The expression levels of *soig* mRNA in the olfactory epithelium have been analysed during several lifecycle stages of lacustrine *O. nerka* and *O. keta*, such as ontogeny, PST and homing. During ontogeny, the expression levels of *soig* mRNA are significantly higher in alevin (juvenile fry) than in embryos at 43 and 60 days after fertilization and then they surge at the PST in lacustrine *O. nerka*.

On the other hand, *soig* mRNA levels in the olfactory epithelium of *O. keta* during homing migration are elevated at the estuary and pre-spawning ground. It is thought that *soig* might be related to olfaction or cell proliferation during both the PST and the final stage of homing.

The olfactory chemoreception is accomplished through binding of the odourant substance to an olfactory receptor (OR) that are reportedly encoded by 100–200 genes (Alioto & Ngai, 2005) in the olfactory epithelium with subsequent propagation of the information to the central nervous system. There are two types of *or* genes namely, main olfactory receptors (*mor*), which are expressed in ciliated olfactory receptor cells and vomeronasal olfactory receptors (VOR, subdivided into V1R and V2R), which are expressed in microvillous olfactory receptor cells. *MOR* genes have also been identified in a number of salmonids (Wickens *et al.*, 2001; Dukes *et al.*, 2004, 2006; Morinishi *et al.*, 2007). Recently, olfactory receptor expression was investigated in different life stages of *S. salar*, demonstrating that seven V2R-like (*olfC*) genes were expressed at higher levels in juveniles (parr and smolts) than in adults (Johnstone *et al.*, 2011). Although many *mors* and *vors* have been identified from several vertebrates owing to the progress of whole genome analysis, many ligands remain uncharacterized. Further intensive molecular biological researches need to be clarified regarding the olfactory chemoreception during imprinting and homing migration in salmonids.

## CONCLUSIONS

This review describes recent studies of the physiological mechanisms of imprinting and homing migration mainly in anadromous *O. keta* from the Bering Sea to Japan as well as lacustrine *O. nerka* in Lake Toya and Lake Shikotsu. Using these model fishes, three different approaches (physiological biotelemetry of homing behaviour, hormonal control mechanisms and olfactory imprinting and discriminating ability) have provided valuable understanding of salmonid imprinting and homing migration. Many questions remain unanswered, however, such as the sensory mechanisms of open-water orientation, the hormonal control mechanisms for sensory systems and the central nervous system, accurate and false homing and seasonal and yearly changes in DFAA composition in natal-stream water. Despite the difficulties of a temporally limited spawning season, research from molecular biology to behavioural biology will provide new concepts for the physiological mechanisms of homing migration in salmonids.

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