



Do all roads lead home? Straying of anadromous brown trout (*Salmo trutta*) in a fjord system

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Do all roads lead home? Straying of anadromous brown trout (*Salmo trutta*) in a fjord system

Kristi Källo

PhD Thesis





Do all roads lead home?

Straying of anadromous brown trout (*Salmo trutta*) in a fjord system

PhD thesis

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May 2023

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Preface and acknowledgements

This thesis is submitted as part of the requirements for the degree of Doctor of Philosophy (PhD) at the Technical University of Denmark (DTU). This thesis was conducted under the supervision of Kim Aarestrup, Henrik Baktoft and Martin Lykke Kristensen; and contains four manuscripts, all reflecting part of the work conducted at DTU between 2019 and 2023. This PhD was co-funded by the EU Interreg “MarGen II” project (#175806), DTU and the Danish Rod and Net Fishing License Funds.

The completion of this thesis would not have been possible without the contribution and help from several lovely people to whom I would like to send my sincerest gratitude:

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Summary

Salmonids are a group of iconic species with high cultural and socio-economic significance, making them one of the most studied group of fish. Salmonids are out-standing for their incredible life-histories, which involve significant physiological and morphological transformations, and migrations that can span over hundreds or even thousands of kilometres. One of the most amazing aspects of salmonid life-histories is their ability to return and spawn in the same area where they were born - a life-history strategy referred to as natal philopatry or natal homing. However, not all salmonids return to their natal river, meaning there is often a proportion of individuals that return to non-natal rivers - a behavioural trait referred to as straying. While straying has important implications for different population parameters, its prevalence, connection to other life-history characteristics and spatio-temporal patterns characterising it remain largely unexplored. To address some of these unknowns, this thesis aimed to investigate various aspects of straying behaviour among anadromous brown trout, also referred to as sea trout, originating from rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk, of which all flow into Mariager fjord, Denmark.

The primary objective of this thesis was to determine the prevalence of straying in Mariager fjord over the course of an individual's life as well as across evolutionary time. This was achieved by PIT tagging juvenile brown trout in river Villestrup during their out-migration in the spring and following their movements from river Villestrup to all of the studied rivers (**MS I, II**); or by PIT tagging juvenile brown trout in the summer in all of the rivers (**MS III**) and following their out-migration in the autumn or spring and tracking their return to any of the rivers. Additionally, as PIT telemetry is not applicable in all systems, suitability of otolith microchemistry in quantifying the level of straying was tested; and the level of gene flow was estimated to determine possible implications of straying on the genetic structure (**MS IV**). As a result, PIT telemetry revealed that on average 37% of sea trout in this system strayed at some point in their life. We further, determined that otolith microchemistry is a viable alternative to PIT telemetry in Mariager fjord. By analysing the otolith microchemistry fingerprints of juvenile brown trout, we were able to assign individuals to their respective river of origin, with an accuracy of 80%. This consequently enabled us to determine that strayers constituted a substantial proportion (43%) of the spawning population in all rivers (**MS IV**), providing further evidence of the prevalence of straying in this system. Genetics analysis further revealed that such high level of straying has left its mark on the genetic structure, as the results of **MS IV** determined that there was a high level of gene flow between

the rivers, to such an extent that all the rivers genetically form just one population. The use of PIT telemetry in **MS I, II and III** further provided the possibility to determine spatio-temporal patterns of straying among sea trout during their first and subsequent returns to freshwater. We found that the majority of the strayers that returned for the first time visited non-natal river(s) only, while repeat spawners displayed high variability in their migratory patterns by migrating both to natal and non-natal rivers, with the migratory patterns changing from one year to the next. Lastly, all of the studies within this thesis contributed to identifying factors that are linked to an individual's likelihood of straying across multiple life stages (juvenile and adult), including developmental status (parr, pre-smolt, smolt) during out-migration (**MS I**), duration of the marine phase (**MS I and III**), and individual length upon return to freshwater as an adult (**IV**).

This thesis provides evidence that straying can be prevalent and is an integral part of many sea trout life-history strategies. Straying has clear effects to the genetic structure and likely to other population parameters, like recruitment. These are important aspects to take into consideration by managers, to ensure the effectiveness of management actions and the sustainability of sea trout populations. While several novel insights into straying were made in this thesis, there are still many aspects that remain unclear and would benefit from additional research, such as fitness related costs, and the prevalence of it in other geographical.

Resumé

Laksefiskene er en ikonisk gruppe af fisk med stor kulturel og socioøkonomisk betydning, hvilket har gjort dem til nogle af de mest studerede fisk i verden. Laksefiskene er særligt bemærkelsesværdige for deres enestående livshistorie, der indeholder betydelige fysiologiske og morfologiske forandringer, samt vandringer, der kan strække sig over hundreder eller endda tusinder af kilometer mellem vandløb og hav. Et af de mest bemærkelsesværdige aspekter af laksefiskenes livscyklus er deres evne til at vende tilbage og gyde det samme sted, som de blev født – en livshistorie-strategi som kaldes "homing". Selvom de fleste laksefisk vender tilbage til deres hjemvandløb, er der nogle individer, der vender tilbage til andre vandløb, hvilket kaldes strejfnings. På trods af strejferes potentielt vigtige betydning for forskellige bestandsparametre, er strejfningsens udbredelse, de rumlige og tidsmæssige mønstre samt forbindelse til andre individuelle livshistorie karakteristika, sjældent undersøgt. For at belyse nogle af disse faktorer, havde denne afhandling til formål at undersøge forskellige aspekter af strejfnings blandt vilde havørreder, stammende fra fire forskellige vandløb i Mariager Fjord, Danmark, herunder Villestrup Å, Kastbjerg Å, Valsgaard bæk og Maren Møllebæk.

Et af det primære formål med denne afhandling var at bestemme strejfraten blandt vilde havørreder i Mariager fjord i løbet af deres liv såvel som deres potentielle konsekvenser over evolutionær tid. Dette blev opnået ved at mærke ørreder med PIT mærker i Villestrup Å under deres udvandring om foråret og derefter følge deres vandringer mellem alle fire undersøgte vandløb (**MS I og II**), samt ved at PIT mærke ørreder om sommeren i alle fire vandløb og følge deres udvandring, samt registrere deres tilbagevending til et af de fire vandløb (**MS III**). Da PIT-telemetry ikke er anvendelig i alle vandløbssystemer, blev egnetheden af ørestensanalyser testet til at kvantificere graden af strejfrater, samt hvilken betydning strejfnings har for niveauet af genflow mellem vandløbene (**MS IV**). I gennemsnit var der hele 37% af de PIT mærkede havørreder, der havde strejft i deres liv. Derudover bekræftede ørestensanalyserne i **MS IV** at fisk, som havde strejft, udgjorde en betydelig del af gydebestanden i alle vandløbene, hvilket understøtter resultaterne fra PIT mærkningerne. En høj strejfrate mellem vandløbene har sat sine spor på den genetiske struktur af havørrederne i de fire vandløb: i **MS IV** blev det dokumenteret, at der var en høj grad af genflow mellem vandløbene, hvilket sandsynligvis betyder, at alle vandløbene udgør én havørredbestand i genetisk forstand. Brugen af PIT telemetry i **MS I, II og III** gav yderligere mulighed for at bestemme rumlige og tidsmæssige mønstre for strejfnings blandt havørreder, der vendte tilbage til ferskvand for første eller efterfølgende gange for at

gyde. Resultaterne viste at flertallet af strejferne i deres første gydesæson vendte kun tilbage til andre vandløb end dem, de selv stammede fra. Havørreder, der vendte tilbage i flere gydesæsoner viste høj variabilitet i deres vandringsmønstre ved at vende tilbage til både deres hjemvandløb og ikke-hjemmehørende vandløb i forskellige gydesæsoner. Det virker altså ikke til at tilbagevendningen til et bestemt vandløb nødvendigvis er låst efter første gydning. Derudover har undersøgelserne i denne afhandling bidraget til at identificere faktorer, der har betydning for den individuelle sandsynlighed for at strejfe i forskellige faser af deres livscyklus, herunder udviklingsstatus (parr, pre-smolt, smolt) under udvandringen fra ferskvand (**MS I**), opholdstid i havet (**MS I og III**) og længde, når havørrederne vendte tilbage til ferskvand som voksne (**MS IV**).

Denne afhandling har dokumenteret, at strejfraten blandt havørred kan være høj og indgår i mange forskellige typer af livshistorier. Strejfningen har betydning for den genetiske struktur og sandsynligvis også for andre bestandsparemetre, såsom rekruttering. Disse resultater er vigtige at tage i betragtning for beslutningstagere, for at sikre effektive forvaltningsforanstaltninger og bæredygtighed af havørredbestanden i forskellige geografiske områder. Selvom denne afhandling har opnået flere nye indsigter i havørredens strejfningsadfærd, er der stadig mange ukendte aspekter, såsom de fitnessrelaterede omkostninger samt niveauet af strejfrater i andre geografiske områder. Det kan derfor anbefales at foretage yderligere undersøgelser indenfor disse områder.

List of original manuscripts:

Manuscript I (MS I)

Källo, K., Baktoft, H., Kristensen, M. L., Birnie-Gauvin, K. & Aarestrup, K. (2022). High prevalence of straying in a wild brown trout (*Salmo trutta*) population in a fjord system. *ICES Journal of Marine Science*, 79: 1539-1547.

Manuscript II (MS II)

Källo, K., Baktoft, H., Birnie-Gauvin, K. & Aarestrup, K. (2022). Variability in straying behaviour among repeat spawning anadromous brown trout (*Salmo trutta*) followed over several years. *ICES Journal of Marine Science*, 79: 2453-2460.

Manuscript III (MS III)

Källo, K., Birnie-Gauvin, K., Baktoft, H. and Aarestrup, K. On the factors affecting migration and straying in brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences*. *In press*.

Manuscript IV (MS IV)

Källo, K., Birnie-Gauvin, K., Baktoft, H., Bekkevold, D., Leshner, C. E., Grønkjær, P., Barfod, G. B., Johnson, R., Whitman, G., Willmes, M., Glessner, J. and Aarestrup, K. Otolith microchemistry combined with genetics reveal patterns of straying and population connectivity in anadromous brown trout (*Salmo trutta*). *Submitted*.

Manuscripts that have been published over the course of this PhD but are not part of this thesis:

Källo, K., Birnie-Gauvin, K., Jepsen, N. and Aarestrup, K. (2023). Great cormorant (*Phalacrocorax carbo sinensis*) predation on adult anadromous brown trout (*Salmo trutta*). *Ecology of Freshwater Fish*, 32: 488-495.

Kristensen, M. L., Olsen, E. M., Moland, E., Knutsen, H., Grønkjær, P., Koed, A., **Källo**, K. & Aarestrup, K. (2021). Disparate movement behavior and feeding ecology in sympatric ecotypes of Atlantic cod. *Ecology and evolution*, 11: 11477-11490.

Objectives

The main focus of this thesis was to investigate straying behaviour in sea trout originating from the rivers flowing into Mariager fjord, Denmark. Straying is often an overlooked aspect of life-histories among salmonids, requiring further research to determine its prevalence in various geographical areas and its connection to other life-history patterns. Consequently, one of the central aspects of this thesis was to determine the prevalence of straying, both over the course of an individual's life (**MS I, II, III and IV**) and across evolutionary time (**MS IV**) in sea trout originating from rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk. Additionally, we aimed to determine the spatial and temporal patterns of strayers for sea trout returning to spawn for the first (**MS I and III**) and subsequent times (**MS II**), as well as factors that affect straying at different life stages (juvenile vs adult; **MS I, II, III and IV**). To address the objectives of this thesis, various methodologies, including PIT telemetry (**MS I, II, III**), otolith microchemistry (**MS IV**) and genetics (**MS IV**) were used, allowing us to assess the advantages and disadvantages of each of the adopted methodologies for studies on straying in salmonids.

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1. Background

“There is a fascination in the wonderful. On the basis of certain facts and absence of facts, there has developed the view that the salmon migrates with almost unerring instinct from some distant feeding place in the sea, where salmon have never or rarely been taken, to its natal river. It is now clear that there is no known instance of a salmon making such journey, whatever might be its mental process in doing so.”

A.G. Huntsman, 1938

1.1 Salmonids

Salmonidae, also referred to as salmonids, is a family of fishes that include among others genus *Salmo*, *Oncorhynchus* and *Salvelinus*. Salmonids are native to the northern hemisphere, although they have also been introduced to other parts of the world, making them globally distributed (Crawford & Muir, 2008; Klemetsen et al., 2003). Salmonids are socio-economically important species that have captivated the attention of scientists for centuries, making them one of the most researched group of fish (Birnie-Gauvin et al., 2019). Salmonids have an interesting life-history, in which case individuals display incredible level of adaptability and resilience to various environmental conditions (Crawford & Muir, 2008; Dempson et al., 2008), take on migrations that can span over thousands of kilometres (Rikardsen et al., 2021; Tucker et al., 2011), and demonstrate high level of variability in life-history strategies (Birnie-Gauvin et al., 2019; Klemetsen et al., 2003).

Salmonids are generally anadromous, meaning individuals hatch in freshwater, which is followed by a migration to the sea (Hendry & Stearns, 2004; Klemetsen et al., 2003; Thorstad et al., 2016). Anadromy is thought to have evolved as a result of a trade-off between fitness (e.g. life-time fecundity) and the risks of mortality (Ferguson et al., 2019; Kendall et al., 2015), as compared to freshwater environments, the sea offers faster growth through greater food availability, while also being accompanied with increased risks of mortality via predation and physiological constraints (Hendry & Stearns, 2004; Jonsson, 1985; Stearns, 1989). Although anadromy is common among salmonids, other migratory forms, for example residency and potamodromy, also exist, with different migratory phenotypes at times co-existing within the same species or population (Ferguson et al., 2019; Kendall et al., 2015; Klemetsen et al., 2003).

According to their breeding strategy, salmonids are divided into two groups: species that spawn only once (semelparous), e.g. pink salmon (*Oncorhynchus gorbuscha*) and coho salmon (*O. kisutch*), or those that spawn several times (iteroparous), e.g. brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*). Semelparous salmonids are also known as “big bang” breeders, because they invest a large proportion of their energetic reserves towards reproduction, while iteroparous species need to preserve energy to return to sea and ensure their survival for potential future breedings (Bowerman et al., 2017; Hendry & Stearns, 2004; Jonsson et al., 1997). While repeat spawning occurs among iteroparous salmonids, the proportion of individuals that actually spawn more than once is often low, due to high mortality during earlier life stages (Jonsson & L’Abée-Lund, 1993; Persson et al., 2022). Nonetheless, repeat spawners are considered to be important for population sustainability, as their life-time reproductive success is often higher compared to the individuals that only spawn once (Birnie-Gauvin et al., 2023; Jonsson & Jonsson, 1999; Serbezov et al., 2012).

1.1.1 Brown trout life cycle and conservation status

The brown trout is outstanding among salmonids for displaying high inter- and intra-population variability in life-history strategies (Jonsson & Jonsson, 2009a; L’Abée-Lund & Hindar, 1990; L’Abée-Lund et al., 1989). Generally, brown trout hatch in freshwater, where they spend one to several years as juveniles, after which a proportion of the population may take on the parr-smolt transformation, known as smoltification, and migrate to sea (Ferguson et al., 2019; Hoar, 1988; Klemetsen et al., 2003). However, not all brown trout migrate to sea. A proportion or entire populations of brown trout may spend their whole life in freshwater or undertake migrations within freshwater, for example between river-lake systems (Ferguson et al., 2019). Further, in sympatric populations where resident and anadromous phenotypes co-exist, the proportion of resident and anadromous trout may vary across geographical areas (Jonsson, 1985; Jonsson et al., 2001). The decision to adopt anadromy or residency is considered to be a phenotypically plastic trait, affected by genetically pre-determined threshold values in traits that are associated with the energetic state of juveniles in their home river during specific decision windows (Ferguson et al., 2019; Wysujack et al., 2009). Although there is a hereditary component associated with opting for residency or anadromy (Ferguson et al., 2019; Jonsson &

Jonsson, 2021), the two phenotypes are not genetically distinct and individuals from either phenotype may breed with each other (Charles et al., 2006).

Anadromous brown trout, often referred to as sea trout, out-migrate from freshwater to the sea throughout the year, with peaks in the spring and autumn (Birnie-Gauvin & Aarestrup, 2019; Jonsson & Jonsson, 2009a). However, the majority of studies investigating out-migration in sea trout have primarily focused on spring migrants, with autumn migrants receiving less attention (Birnie-Gauvin et al., 2019). This likely stems from the assumption that autumn migration is maladaptive (Riley et al., 2008), as lower return rates among autumn out-migrants have been documented (Jonsson & Jonsson, 2009a). However, more recent investigations from rivers Gudsø in Denmark and Burrishoole in Ireland have shown that this may vary across geographical areas, as autumn migrants may also successfully return to freshwater to the same extent as spring migrants (Birnie-Gauvin & Aarestrup, 2019; Wynne et al., 2023). Subsequent to out-migration from freshwater, the length of the marine phase may vary significantly, both within and among populations, ranging from a few weeks to several years (del Villar-Guerra et al., 2019; Jonsson & Jonsson, 2009a; Jonsson & L'Abée-Lund, 1993).

After the marine phase, sea trout migrate back to freshwater to spawn. Similar to out-migration, the timing of return to freshwater varies between individuals and populations. For example, part of the spawning population may already return in early summer, while the rest may return during the autumn (Birnie-Gauvin et al., 2021; Jonsson & Jonsson, 2009a), shortly before or during the spawning season, which takes place from early autumn to early spring, depending on latitude (Campbell, 1977; Gortázar et al., 2007; L'Abée-Lund et al., 1989). Further, brown trout are iteroparous, meaning individuals may spawn for multiple times (Klemetsen et al., 2003). However, the proportion of repeat spawners is usually low compared to the overall number of individuals that return (Aarestrup et al., 2015; del Villar-Guerra et al., 2019; Kristensen et al., 2019a), but this also varies in different geographical areas (Jonsson & L'Abée-Lund, 1993).

Sea trout, like other diadromous fishes, are vulnerable to climate change and anthropogenic activities (Gosset et al., 2006; Jonsson & Jonsson, 2009b). Sea trout utilize and migrate through multiple habitats at different life stages, which exposes them to a diverse set of environmental conditions and stressors (Nevoux et al., 2019; Reid et al., 2019). Important factors associated with the decline in abundance of sea trout in multiple areas (Clavero et al., 2017; Poole et al., 1996) include loss of habitat, through damming and straightening of rivers (Belletti et al., 2020; Birnie-Gauvin et al., 2018), environmental

pollution and climate change (Jonsson & Jonsson, 2009b). While sea trout continues to be one of the most researched fish globally, there are several aspects of sea trout life-history that remain poorly understood, which emphasises the need to further our understanding of different life-history strategies sea trout may adopt and factors that affect individuals at different life stages in various geographical areas. Comprehensive understanding of the aforementioned aspects may prove extremely important, to mitigate the potential negative effects to the sustainability of sea trout populations, particularly during times when fish populations continue to be under increasing pressure due to climate change and anthropogenic activities.

1.2 Natal philopatry

1.2.1 What is natal philopatry?

Understanding geographical borders of populations and the connectivity between them is essential in making informed management and conservational decisions (Begg et al., 1999; Kerr et al., 2014). While identifying population boundaries can be challenging in many taxa due to complex structuring between them (Coates et al., 2018; Nordeide et al., 2011; Palsbøll et al., 2007), some taxa instead exhibit a life-history strategy known as natal philopatry. Natal philopatry is a strategy to return to breed in the same area where you originated from, which results in distinct genetic structuring between populations (Knutsen et al., 2018; Stiebens et al., 2013). Further, natal philopatry serves as an evolutionary mechanism through which individuals increase their fitness by having higher likelihood of finding suitable partners and habitats during reproduction, as well as it leads to the development of local adaptations (Table 1; Keefer & Caudill, 2014; Mobley et al., 2019). Natal philopatry is prevalent in several vertebrate taxa, including fishes, birds, mammals and amphibians (Greenwood, 1980; Lohmann et al., 2013).

Among salmonids, natal philopatry, also referred to as natal homing, is a life-history strategy whereby individuals return to breed in the same river or tributary where they had hatched (Keefer & Caudill, 2014). In the animal kingdom, salmonids are considered outstanding in natal homing behaviour, as a high proportion of individuals from a population display tendencies to return to their natal river (Candy & Beacham, 2000; Jonsson & Jonsson, 2014; Quinn, 1993), at times with great accuracy, for example to the same stretches of river where they had hatched (Hamann & Kennedy, 2012; Vähä et al., 2007).

Table 1. Glossary of frequently used terms in this thesis

Term	Definition
Natal philopatry	The return of individuals to breed in the same area where they were born. Among salmonids, this term is also referred to as natal homing.
Straying	The return of an adult salmonid to non-natal river or tributary, presumably with the aim to spawn.
Olfactory imprinting	Process during which individuals learn the olfactory cues of the natal area which are subsequently used to identify the route back to the natal area as adults.

1.2.2 Mechanisms of natal homing in salmonids

Natal homing is a complex behavioural and physiological process, where several mechanisms that are interconnected with each other, have an effect on its success. The main processes involved in natal homing are (1) natal imprinting as juveniles to the cues of the natal river (Hasler et al., 1978; Lema and Nevitt, 2004), (2) use of Earth's magnetic field during long-distance migration (Lohmann et al., 2008); and (3) migration back to the breeding ground based on the cues learned as juveniles (Armstrong et al., 2021). While several aspects of these aforementioned processes have been a subject to intensive research, especially those related to natal imprinting as juveniles (Dittman & Quinn, 1996; Hasler & Scholz, 1983), the mechanisms of natal homing are still not fully understood.

The primary process of natal homing is natal imprinting, during which juveniles learn the cues of their natal river, which are subsequently used to recognize and return to it as adults. There are three main theories concerning the general mechanisms of natal imprinting: (1) Hasler and Wisby (1951) suggested an olfactory imprinting theory, where salmonids imprint to chemicals, likely to specific organic compounds, in their natal river; (2) Nordeng (1971) suggested that juveniles instead imprint to the pheromones of their relatives and (3) Bett & Hinch (2016) combined the two previous hypotheses by suggesting that natal imprinting is a hierarchical process, during which olfactory cues imprinted as juveniles form the primary cues, pheromones from relatives and conspecifics secondary cues and non-olfactory environmental waypoints as the tertiary cues used by salmonids to locate their natal breeding grounds.

While the true mechanisms of natal imprinting are still subject to discussion, there is compelling evidence, that imprinting to olfactory cues of the natal river is an important aspect of natal imprinting, if not the primary mechanism of it (Armstrong et al., 2021; Hasler et al., 1978). Notably, specific chemical compounds, mainly amino acids, have been named as essential cues that salmonids learn during natal imprinting (Yamamoto et al., 2008). For example, Shoji et al. (2003) showed in an experiment that chum salmon (*O. keta*) can recognize specific amino acids derived from their natal river from artificial water and navigate in freshwater according to them. Imprinting to amino acids has subsequently been documented in similar experiments in pink, sockeye (*O. nerka*) and masu salmon (*O. masou*) (Yamamoto et al., 2008), indicating their importance in the olfactory imprinting process in genus *Oncorhynchus*. While no such experiment has been conducted in the genus *Salmo*, Johnstone et al. (2011) has shown increased expression of genes related to olfaction, which have further been hypothesised to act as amino acid receptors, during the juvenile life stages in Atlantic salmon and may indicate that amino acids are important cues in the *Salmo* genus as well.

The endocrine system is considered essential in the process of olfactory imprinting. More specifically, elevations in plasma thyroid hormones during the juvenile life stage, are known to increase the production of olfactory receptor neurons (Lema & Nevitt, 2004), which are essential in sending signals to the olfactory nerve and subsequently to the brain (Bett & Hinch, 2016). This indicates that changes in plasma thyroid levels are directly linked to the ability of individuals to learn the olfactory cues of their natal river. During the early years of studies into olfactory imprinting, it was thought that olfactory imprinting mainly took place over the smoltification period, when the largest increases in thyroid hormones take place (Dittman & Quinn, 1996; Hasler & Scholz, 1983; Hasler et al., 1978). This was empirically supported by the results of transplantation studies, where juvenile salmonids that had been transported to a new river shortly prior to smoltification returned to a high degree to the river of out-migration, while individuals that were released post-smoltification did not (Hasler & Scholz, 1983). More recent investigation into olfactory imprinting, however indicates that, even though smoltification is important, olfactory imprinting takes place over an extended period as juveniles (Armstrong et al., 2021). More precisely, surges in thyroid hormones, which affect proliferation of olfactory cells already at low elevations (Lema & Nevitt, 2004), are known to occur throughout the juvenile phase, starting as early as the embryonic stages (Boeuf et al., 1989; Dickhoff et al., 1982; Sullivan et al., 1987). There is further empirical evidence that salmonids that have been imprinted to artificial odours during embryonic stages

can recognize them and orientate according to the odours they experienced during that period (Armstrong et al., 2021; Havey et al., 2017), further pointing towards the importance of olfactory imprinting over extended period as juveniles.

Less is known about the mechanism that salmonid use to navigate during the marine phase. It has been hypothesised that salmonids, like several other species that undertake long migrations, use the spatial variation in Earth's magnetic field as a map to guide them between feeding and breeding areas (Lohmann et al., 2008; Putman et al., 2013). More precisely, long distance migrants have the ability to sense the intensity and inclination of Earth's magnetic field, and consequently orientate according to it. Compared to imprinting to olfactory cues, which is mainly considered to be affected by extrinsic factors, geomagnetism and the magnetic map individuals follow during their marine migration is likely hereditary (Putman et al., 2014). The use of geomagnetism during marine migrations has been shown in Atlantic salmon and several *Oncorhynchus* species (Putman et al., 2013; Scanlan et al., 2018), but less is known about its importance in shorter distance migrants, like sea trout. Sea trout are commonly coastally orientated (Eldøy et al., 2015; Kristensen et al., 2019b), that generally do not undertake long marine migrations, like their close relative, Atlantic salmon (Rikardsen et al., 2021), leaving therefore room to the question how well geomagnetism is developed among them.

1.3 Study area

In this thesis, straying behaviour of sea trout originating from four different rivers, Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk, flowing into Mariager fjord was investigated (Figure 1). Mariager fjord is situated on the east coast of Jutland, Denmark, and it is connected to the Kattegat from the east. The salinity in the fjord ranges between 12 and > 20 ‰ and the depth ranges between 1 and 30 m. The fjord is approximately 40 km long and 2 km wide (Fallesen et al., 2000).

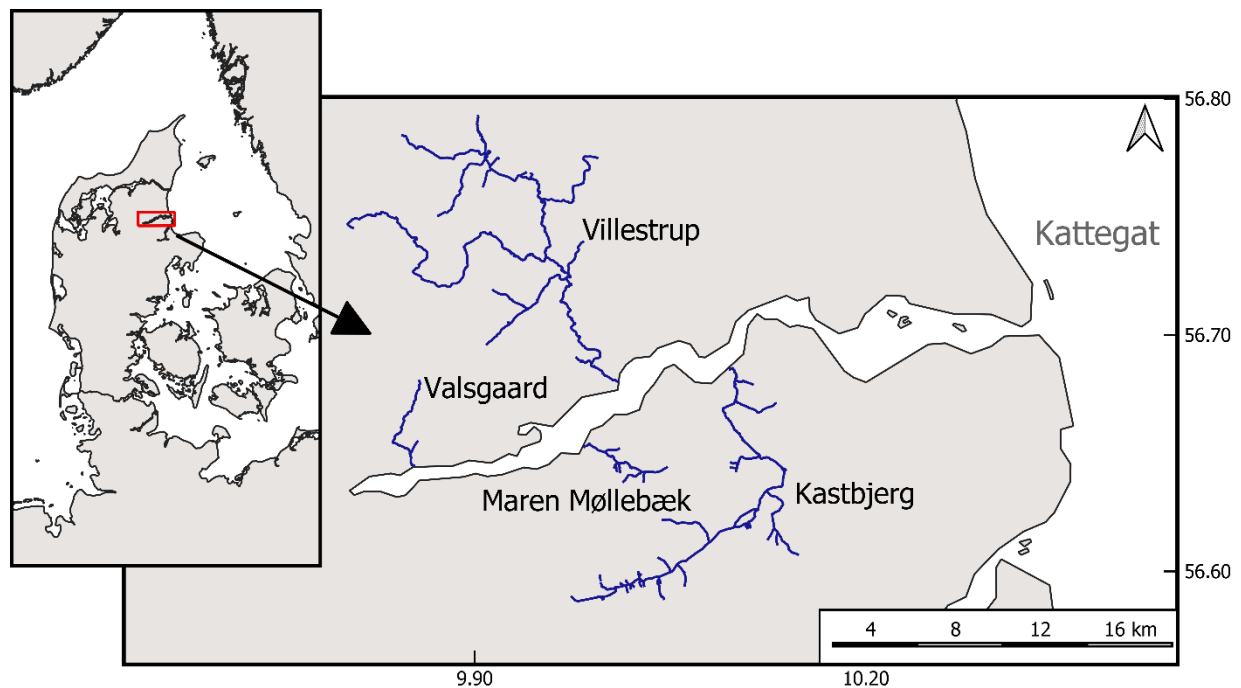


Figure 1. Study area of this thesis, where patterns of natal homing and straying behaviour were investigated among sea trout originating from rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk.

River Villestrup is the largest river (according to flow) that flows into Mariager fjord and it is located on the northern side of the fjord. It is approximately 21 km long and has a stable flow due to groundwater influx. River Kastbjerg, which is situated on the southern side of the fjord, is the second largest river in Mariager fjord. It is approximately 21 km long, and has an average annual flow of $0.8 \text{ m}^3\text{s}^{-1}$. Rivers Valsgaard and Maren Møllebæk are significantly smaller, ~ 7 and 5 km long, and are situated on the northern and southern side of the fjord, respectively (see Table 2 for further details). Even though, there

is no exact estimates on the size of the breeding population for all the rivers studied in this thesis, river Villestrup has likely the largest, followed by Kastbjerg, Valsgaard and Maren Møllebæk.

Table 2. Main characteristics of rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk (Table adjusted from **MS III**).

	Villestrup	Kastbjerg	Valsgaard	Maren Møllebæk
Length (km)	21	21	7	5
Width (m)	4 – 12	3 – 8	0.5 – 3	0.5 – 2
Depth (m)	0.5 – 3	0.3 – 2	0.1 – 1	0.01 – 0.3
Mean annual discharge \pm SD (m^3s^{-1})	1.6 ± 0.3	0.8 ± 0.3	0.1 ± 0.02	NA
Mean annual temperature \pm SD ($^{\circ}\text{C}$)	8.9 ± 3.0	9.9 ± 4.4	9.1 ± 3.2	9.8 ± 2.6

2. Straying

Although natal homing is considered prevalent among salmonids, not all individuals always return to their natal river, some instead enter non-natal rivers to breed, a behavioural trait referred to as straying (Table 1; Degerman et al., 2012; Quinn, 1993). Straying is an evolutionary important mechanism, complimentary to natal homing, through which individuals (re)colonize new habitat (Hendry & Stearns, 2004; Keefer & Caudill, 2014; Østergaard et al., 2003). It further facilitates gene flow between populations (Bekkevold et al., 2020; Hess & Matala, 2014; Massa-Gallucci et al., 2010), thereby increasing a population's resilience to environmental change. Straying may also have more immediate effects on both the donor and the recipient population, for example by affecting the demographic structure of the spawners and recruitment (Bett et al., 2017; Bowler & Benton, 2005).

Straying and natal homing are the opposite sides of the same coin, therefore, similar to natal homing, the mechanisms and proximate causes for straying are not fully understood, making it difficult to determine whether straying is the result of a failure by salmonids to recognize and return to their natal river or an evolutionary strategy that maximizes individual fitness (Keefer & Caudill, 2014). While hatchery origin (Candy & Beacham, 2000; Ford et al., 2015) and pollution (Moore et al., 2007) have been associated with reduced natal homing abilities, likely due to impaired olfactory learning or recognition, there is also evidence suggesting that decision based processes aimed at maximizing individual fitness can affect straying rates. For example, poor habitat quality (Ford et al., 2015; Østergaard et al., 2003) and individual life-history characteristics, which are associated to fitness related traits (e.g. sex; Hard & Heard, 1999), have been linked to increased straying rates. Moreover, the drivers and causes for straying may even vary among individuals of the same populations (Ford et al., 2015).

2.1 Prevalence of straying

2.1.1 Qualitative assessments of straying

Straying in salmonids has been studied to varying degrees in different species and regions (Keefer & Caudill, 2014; Quinn, 1993), thus our understanding of the prevalence and mechanisms that drive straying differs depending on taxa and location. The majority of studies investigating straying in

salmonids have been conducted in genus *Oncorhynchus*, with the genus *Salmo* receiving considerably less attention (Birnie-Gauvin et al., 2019). Straying has further been prevalently documented qualitatively using genetic methods, by determining the level of gene flow between populations (Bekkevold et al., 2020; Massa-Gallucci et al., 2010; Walter et al., 2009). This means that in areas where natal homing tendencies are strong, clear genetic differences between populations form, while in areas of increased connectivity between populations aka straying, such differences diminish. As strayers do not always successfully spawn in non-natal rivers (Dionne et al., 2008; Mobley et al., 2019), estimating gene flow between populations provides the possibility to investigate patterns of successful reproductive straying over evolutionary time.

The results from population genetic studies indicate that the level of successful reproductive straying varies between salmonid populations. Depending on species and geographical areas, clear genetic differences between rivers (Dionne et al., 2008; Lin et al., 2008) or even tributaries of the same watershed (Kitanishi et al., 2009; Vähä et al., 2007) and low levels or no genetic differences between populations have been documented (Bradbury et al., 2014; Ikediashi et al., 2018). Similar genetic patterns also characterize brown trout populations. For example, in a large-scale study in northern Europe, Bekkevold et al. (2020) documented that in some areas there were clear genetic differences among nearby populations, while in others there were not, indicating that the level of reproductively successful straying differed across the region. Similar patterns have further been documented in other studies on a smaller scale (Massa-Gallucci et al., 2010; Östergren et al., 2012).

In **MS IV**, Single Nucleotide Polymorphism (SNP) markers were used to determine the level of gene flow in rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk (Figure 1). We found that there was a high level of gene flow among individuals originating from these rivers, so much so that it appears the rivers genetically make up one population. This is, therefore, a clear indication that sea trout in this system migrates to non-natal rivers, where they are able to successfully reproduce. Further, the results of **MS IV** indicated that the genetic structure of sea trout in Mariager fjord has been stable over time, since the genetic structure of sea trout in river Villestrup did not change significantly over a 10-year period. Temporal stability in the genetic structure of sea trout populations seems to also be prevalent in other geographical areas (Ayllon et al., 2006; Bekkevold et al., 2020), however not always, notably in unstable environments and over longer time periods (Østergaard et al., 2003).

2.1.2 Quantitative assessments of straying

In contrast to qualitative assessments, quantitative estimates on the prevalence of straying at the species and population level are less commonly reported (Birnie-Gauvin et al., 2019; Keefer & Caudill, 2014). The majority of these studies have been conducted on hatchery salmonids that are considered to have impaired natal homing abilities, resulting in increased straying (Ford et al., 2015; Jonsson & Jonsson, 2014; Jonsson et al., 2003), with wild salmonids receiving less attention. Nevertheless, quantitative estimates indicate that straying rates may be high or low, and vary depending on the species and populations (Table 3). However, differences in methodology between studies and a limited number of estimates make it difficult to generalize findings and draw conclusions about the mechanisms that drive straying.

A primary objective of this thesis was to provide quantitative estimates of straying for individuals originating from four rivers flowing into Mariager fjord. More precisely, we used Passive Integrated Transponder (PIT) telemetry to estimate the rate of straying among sea trout tagged as juveniles in river Villestrup migrating to rivers Kastbjerg, Valsgaard and Maren Møllebæk (**MS I** and **II**) or among individuals tagged in all four rivers possibly straying *into* each of the four rivers (**MS III**). We found that the average rate of straying was 37%, with no significant differences in the level of straying between individuals originating from different rivers (**MS III**). Additionally, in **MS IV**, the level of straying was estimated using otolith microchemistry by determining the proportion of the spawning population that strayers constituted. It was estimated that in average 43% of mature sea trout had returned to non-natal rivers, with no significant differences in the proportion of strayers and natal homers between the rivers, indicating that strayers make up a significant proportion of the spawning population in all of the rivers in Mariager fjord.

To our knowledge, the straying rates presented in **MS I**, **III** and **IV** are the highest estimates of straying between rivers documented in wild sea trout throughout its distribution range (Table 3). However, high straying rates have been documented among rivers flowing into the Bay of Biscay, where 0-35% of sampled sea trout were determined to have originated from a non-natal river (Masson et al., 2018). High straying rates have further been documented among sea trout straying between the tributaries of the same river system (47%; Mikhveev et al., 2021) as well as among hatchery origin sea trout returning to Swedish coastal rivers (5-46 %, Degerman et al., 2012).

There was indication for some temporal variation in straying rates in Mariager fjord, at least when investigating rates of straying among sea trout tagged in river Villestrup over four years of tagging. More precisely, in **MS I** and **III** the rate of straying ranged between 34 and 54%, indicating that while there seems to be some variation in the rate of straying from one year to the next, overall straying remained high throughout the study period. Temporal variability in straying rates is not surprising, as it has been previously documented in genus *Oncorhynchus* (Quinn & Fresh, 1984) and Atlantic salmon (Jonsson et al., 2003).

Table 3. Previously reported quantitative rates of straying* among wild and hatchery origin salmonids.

Species	Origin	Straying rate	Reference
<i>Salmo spp.</i>			
Brown trout	Wild	1.6 %	Jonsson & Jonsson (2014)
Brown trout	Wild	16 %	Berg & Berg (1987)
Brown trout	Wild	0-35 %	Masson et al. (2018)
Brown trout	Wild	47 %	Mikheev et al. (2021)
Brown trout	Wild	12-55 %	MS I, III
Brown trout	Hatchery	7.0 %	Jonsson & Jonsson (2014)
Brown trout	Hatchery	5-46 %	Degerman et al. (2012)
Atlantic salmon	Wild	6 %	Jonsson et al. (2003)
Atlantic salmon	Hatchery	15 %	Jonsson et al. (2003)
<i>Oncorhynchus spp.</i>			
Chinook salmon	Wild	13 %	Hamann & Kennedy (2012)
Chinook salmon	Hatchery	0-4 %	Candy & Beacham (2000)
Chinook salmon	Hatchery	10-27 %	Quinn et al. (1991)
Chinook salmon	Hatchery	0-55 %	Westley et al. (2013)
Coho salmon	Wild	15-27 %	Shapovalov & Taft (1954)
Coho salmon	Hatchery	<1 %	Westley et al. (2013)
Sockeye salmon	Wild	5-9 %	Lin et al. (2008)
Steelhead (<i>O. mykiss</i>)	Hatchery	0-2 %	Westley et al. (2013)

*There may be differences among the presented studies in the methods used to estimate the rates of straying, as well as in the spatio-temporal scale over which individuals were determined to have strayed

2.2 Spatial and temporal patterns of straying

Spatial patterns of straying in salmonids have generally been investigated from the perspective of individuals straying from one donor population into multiple recipient populations, with the latter being often monitored with varying sampling intensities, likely resulting in under-estimation of the level of straying (Berg & Berg, 1987; Jonsson et al., 2003). Straying data have often been dependent on the reporting by anglers, making it challenging to infer detailed information about temporal and spatial patterns of straying, as it may be unclear when the fish may have entered the river, if they have previously been to other rivers or if they had strayed or homed in previous spawning seasons. Further, individuals migrating to non-natal rivers have been grouped as strayers independent of the time of year or at various time periods they were documented in non-natal rivers (Degerman et al., 2012; Jonsson et al., 2003). Consequently, knowledge about the temporal and spatial patterns of straying, especially in sea trout, is limited.

In this thesis temporal and spatial patterns of straying were investigated among sea trout returning to freshwater for the first (maiden spawners; **MS I** and **III**) and those returning for multiple times (repeat spawners; **MS II**), of which the latter has never been investigated in detail among sea trout before. Maiden and repeat spawners are both crucial for the sustainability of the population, as the former often make up the majority of the spawning population (L'Abée-Lund et al., 1989), while the latter may contribute to the recruitment proportionally more compared to the maiden spawners, thus significantly contributing to the temporal stability of recruitment in a given river (Birnie-Gauvin et al., 2023; Jonsson & Jonsson, 1999; Serbezov et al., 2012).

In **MS I** and **MS III** we documented that strayers that returned to freshwater for the first time displayed two main patterns: (1) the majority of the strayers only returned to non-natal rivers, out of which most were in the non-natal river during the spawning season, while there was also (2) a smaller proportion of strayers that were detected both in their natal and a non-natal river, with the majority being in their natal river during the spawning season. While migration between natal and non-natal rivers has been documented to occur among sea trout returning to freshwater for overwintering (however not within the same season; Jensen et al., 2015), migration between natal and non-natal rivers just prior or during the spawning season (as documented in **MS I** and **MS III**) has never been reported among sea trout returning to freshwater presumably with the aim to spawn. However, the pattern that some individuals

return directly to their spawning grounds, while the others take non-directional routes has been documented in sea trout (Aarestrup & Jepsen, 1998; Finstad et al., 2005) and other salmonids (Keefer et al., 2008) within the same or between multiple river system(s) (Peterson et al., 2016), possibly implying there is a divergence in migratory patterns, which is common among salmonids returning to spawn in various spatial scales.

To date, our understanding of how straying patterns may vary among repeat spawning salmonids over the course of their life is very limited. This may be the case because majority of the studies investigating straying among salmonids have been conducted in Pacific salmonids, which are generally semelparous species. Thus, do repeat spawning salmonids that stray upon their first spawning also stray upon their second and third (and so on) spawning? Or do the patterns of straying change over the lifetime of an individual? **MS II** is the first comprehensive study to investigate the patterns of straying among iteroparous salmonids by following the same individuals over multiple spawning seasons, which consequently significantly contributes to our understanding of possible implications of straying. The results of **MS II** show that straying among repeat spawners is complex and subject to change over an individual's life, possibly implying it is a plastic life-history trait. More specifically, two contrasting strategies were documented among repeat spawning strayers originating from River Villestrup: (1) there was a minority of strayers that returned to the same non-natal river throughout their life (spawning site fidelity), while (2) the majority of repeat spawning strayers displayed high variability in their migratory patterns. The latter group migrated between natal and non-natal rivers, with the migratory patterns changing from one year to the next (Figure 2). These migratory patterns however were not completely random, as there was some indication that the more times an individual returned to spawn, the more likely it was to return to its natal river. While, very little is known about the migratory patterns of repeat spawning strayers, Jonsson et al. (2018) has documented decreased straying among repeat spawners compared to maiden spawners among Atlantic salmon. While Jonsson et al. (2018) did not explore straying behaviour within the same individuals across years, but rather only compared maiden and repeat spawners within a single year, these results seem to coincide with the results of **MS II**.

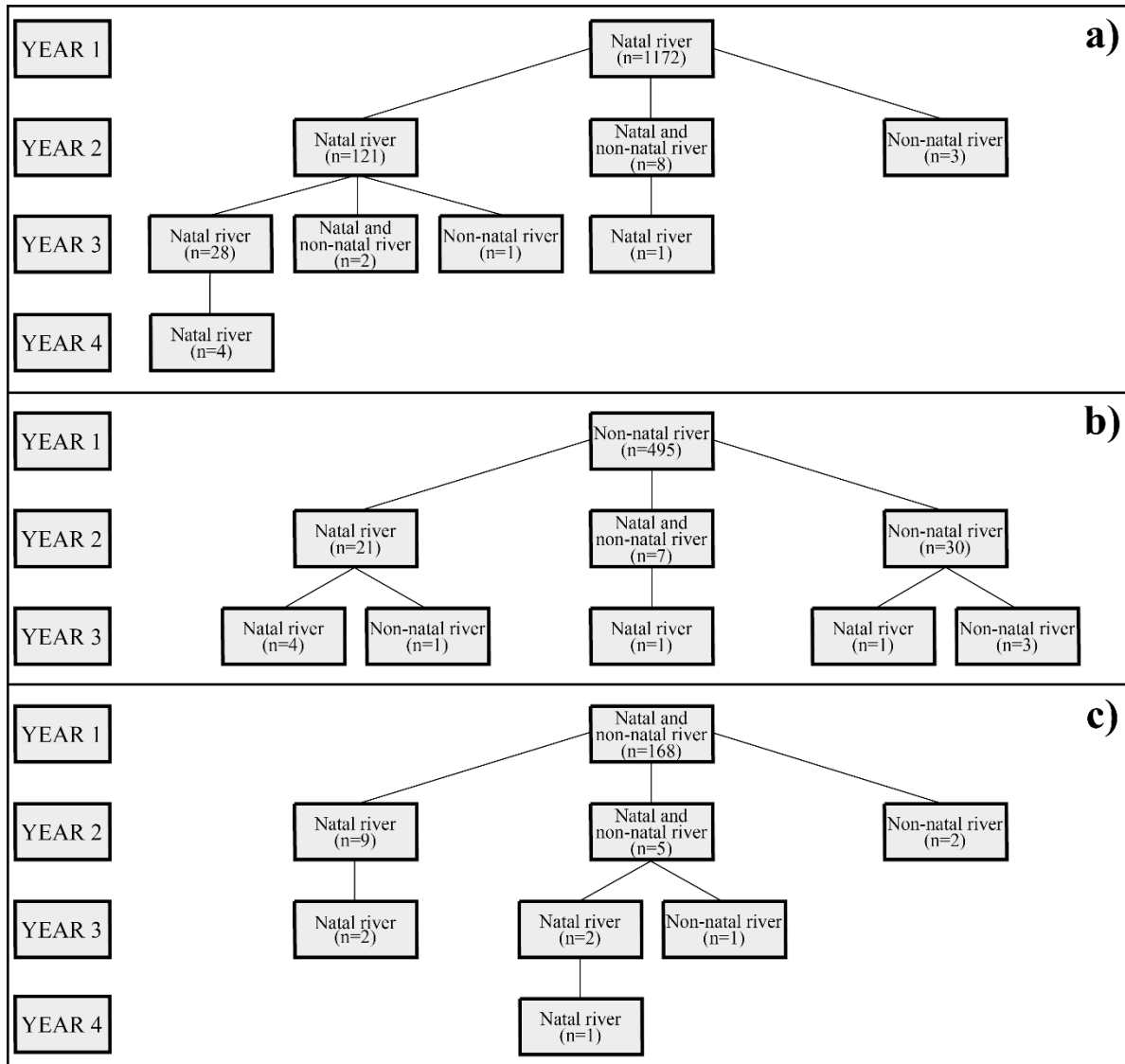


Figure 2. Migratory patterns of sea trout returning to freshwater across years depending on whether they had returned to their natal (a), non-natal (b) or both to natal and non-natal (c) river(s) during their first year of return (from **MS II**).

The rivers that individuals strayed to in Mariager fjord were similar across studies, with the two larger rivers, Villestrup and Kastbjerg, being the prominent recipient rivers for strayers (**MS I, III, IV**). While straying to the rivers closest to their natal river has been commonly documented (Berg & Berg, 1987; Jonsson et al., 2003; Ozerov et al., 2017), there are also studies documenting increased straying towards the larger rivers (Degerman et al., 2012; Unwin & Quinn, 1993), which is in agreement with our findings. The genetic results of **MS IV** further indicated that straying (at least reproductively successful straying) of Mariager fjord sea trout is to a large extent contained within the fjord. More specifically, we

found clear genetic differences between individuals originating from the rivers inside the fjord and those originating from outside of the fjord, which indicates low levels of gene flow between these two areas. While it is expected to have lower genetic connectivity between the populations within and outside of the fjord due to expected decrease in straying with increasing distance from the natal river, the rivers outside of the fjord in the present study are still within the distance (~60 km) over which straying has been documented to be prevalent (Bekkevold et al., 2020; Jonsson et al., 2003). This may therefore suggest that the fjord acts as a natural barrier to gene flow between the rivers within and outside of the fjord. However, it cannot be excluded that the Mariager fjord sea trout regularly attempt to spawn in the rivers outside of the fjord but are unsuccessful.

2.3 Factors linked to patterns of straying

The evolutionary reasons for straying are quite clear, it facilitates gene flow between populations, thereby increasing population resilience to environmental change, and is a mechanism through which individuals (re-)colonize new areas (Keefer & Caudill, 2014). However, the proximate causes for straying and the fitness related costs and benefits associated with it remain unclear and are often difficult to identify. Regardless, various intrinsic and extrinsic factors have been connected to individual likelihood of straying, including environmental conditions (Bendall et al., 2005; Bett et al., 2017; Moore et al., 2007), individual origin (wild vs stocked; Jonsson et al., 2003; Jonsson & Jonsson, 2014) and characteristics (e.g. sex; Hard & Heard, 1999), with the proximate reasons why some individuals stray and others do not likely varying in different species and populations (Ford et al., 2015; Westley et al., 2013). To further elucidate the underlying mechanisms of straying, investigations into the extrinsic and intrinsic factors linked to its prevalence are necessary. The use of different methodologies and investigations across life-stages (juvenile vs adults) in this thesis have provided us with the ability to resolve some of these unknowns.

In **MS I** we documented that individual likelihood of straying was lower for individuals that migrated as parr in the spring, compared to those that out-migrated as pre-smolts and smolts. These results provide additional empirical evidence that the completion of smoltification prior to out-migration is not necessary for an individual's ability to home, as natal imprinting likely occurs over an extended period during the juvenile phase (Armstrong et al., 2021), enabling non-smoltified sea trout to successfully return to their

natal river. Individual likelihood of straying, however, varied between the developmental groups (parr, pre-smolt, smolt) depending on the day of out-migration and the length of the individuals during out-migration, independently. These effects were most pronounced in parr, with longer individuals and those out-migrating later having reduced likelihood to stray (Figure 3). It is unclear how the developmental status in connection to length and day of out-migration interact with each other to affect the likelihood of straying, however as natal imprinting and smoltification are complex processes, interconnected with each other, they are likely affected by various extrinsic and intrinsic factors. We further investigated whether out-migration phenology (spring vs autumn migrants), which was related to individual likelihood to return, in a river specific manner in this system, had an effect on individual likelihood of straying and found no such connection (**MS III**).

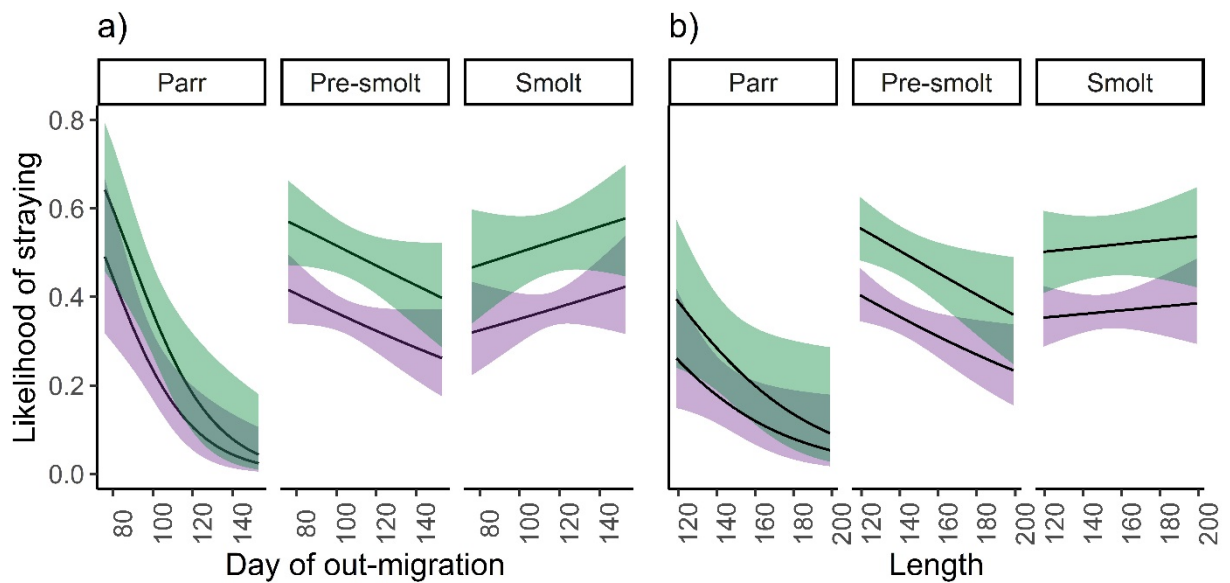


Figure 3. Likelihood of straying differs between developmental groups (parr, pre-smolt, smolt), which was further linked to (a) day of out-migration and (b) length as juveniles that were tagged in 2015 (purple) and 2016 (green) (from **MS I**).

In both **MS I** and **MS III**, time spent at sea after out-migration as a juvenile, was identified as an important variable affecting the likelihood of straying, with individuals that spent less time at sea straying more often. This contradicts the findings of Jonsson et al. (2003) and Quinn et al. (1991), who documented increased straying the longer individuals stayed away from freshwater. The authors suggested that (1) strayers that spent longer time at sea may have forgotten the cues of their natal rivers, (2) there may have been changes in water chemistry over the years, impeding natal river recognition or (3) larger

individuals having chosen other, possibly bigger rivers, to return to. The opposite pattern documented in **MS I** and **III** was hypothesised to be linked to differences in life-history strategies between sexes, based on the documentation that males, in general (Hamann & Kennedy, 2012; Turcotte & Shrimpton, 2020), as well as younger males (Hard & Heard, 1999), have a higher likelihood to stray. The findings of **MS IV** seem to contradict this hypothesis, as there were no differences in the proportion of strayers between the sexes.

MS IV further documented that individual length upon return to freshwater as an adult had an effect on individual likelihood of straying, which varied depending on the river individuals originated from. More specifically, smaller individuals from river Villestrup were more likely to stray, while a positive relationship between length and straying likelihood was documented in rivers Kastbjerg and Valsgaard, with longer individuals being more likely to stray. The causes for these differences remain unclear, however demonstrate that patterns of straying may differ between individuals that share the same marine environment, indicating that river specific factors are likely at play here.

In conclusion, the findings of this thesis suggest that for Mariager fjord sea trout straying seem to be an inherent part of their life-history. The studies of this thesis found straying to be associated with various life-history characteristics at different life stages, as straying was documented in spring and autumn out-migrants, maiden and repeat spawners, females and males, small and large juveniles and adults, as well as in individuals that out-migrated as parr, pre-smolt and smolt. Consequently it may be hypothesised whether straying in this system acts as a life-history strategy aimed at maximizing individual fitness, instead of a failure to recognise and return to their natal river. However, as the fitness related costs of straying remain unclear, further investigation is needed to compare the life-time fitness of strayers to that of natal homers.

2.4 Methods to study the prevalence and patterns of straying

2.4.1 Telemetry

With the advancement of technology and production of smaller tags, telemetry has become a popular tool in fisheries science (Cooke et al., 2013; Hussey et al., 2015; Thorstad et al., 2013). It provides the possibility to obtain detailed information on fish behaviour, enabling scientists to follow the movement of fish with high spatial and temporal resolution. To date, several different tags have been produced to

fit different species, environments and scientific needs (Cooke et al., 2013). In **MS I, II** and **III**, PIT tags were used to study the straying behaviour of sea trout. PIT tags do not have an internal battery and instead rely on fish moving in close proximity to a PIT antenna system, during which the tag will get energized, so it can send a unique ID to the antenna. The ID and timestamp are then recorded by the antenna. Thus, it is possible to register the timing of movement for fish at known locations, making PIT tags well suited to use on fish that take on predictable movements between known habitats, for example like salmonids that migrate out of rivers as juveniles and subsequently return to it as adults.

MS I, II and **III** demonstrated that PIT tags are well suited for studying straying behaviour of sea trout. PIT telemetry is cost effective, especially compared to other forms of electronic tagging, as tags are relatively cheap, allowing to tag large number of individuals. Further, as PIT tags do not rely on a battery to document movements of fish, they have, in principle, an indefinite life-span, as long as they do not get damaged or have a malfunction. These characteristics have allowed us to tag a total of 25 027 juvenile trout and follow them for up to 5 years post-tagging, within the framework of this thesis. This has resulted in multiple novel insights regarding sea trout life-history strategies, including repeat spawning strayers, an understudied life-history aspect among sea trout (Birnie-Gauvin et al., 2019).

The ability to tag a high proportion of out-migrating individuals has been essential in the studies presented in this thesis, as the number of sea trout returning to freshwater as adults is often low. The return rate of sea trout has been documented to range between 8.3 - 24 % in this system (**MS III**; del Villar-Guerra et al., 2019) and ~1 - 35 % in other systems (Jonsson & Jonsson, 2009a), with even fewer fish detected in non-natal rivers (Table 3) and returning for multiple spawning seasons (Jonsson & L'Abée-Lund, 1993). For example, among the fish tagged in **MS I** and **MS II**, 8.5% of the tagged individuals returned to freshwater, with 1% of tagged individuals returning for multiple years (repeat spawners) and 0.4 % of tagged individuals being repeat spawning strayers. Thus to be able to connect migratory patterns to different life-history characteristics or to other measured variables, it is necessary to tag a large number of fish to have a sufficient sample size to ensure trustworthy significance estimates. This is especially important in studies on sea trout, given that they are highly variable in their life-history traits (Birnie-Gauvin et al., 2019; Klemetsen et al., 2003), which may make it difficult to determine true biological signals from variance in the data.

Although PIT telemetry allowed us to investigate several novel behavioural patterns in sea trout, it may not be suitable in all systems or for all populations (Cooke et al., 2013). Rivers in Mariager fjord are

relatively narrow and shallow, which make it possible to mount antennas at the outlet, while also retaining high detection efficiencies when fish swim through the system (**MS III**). In deeper rivers, or those with high flow, PIT telemetry may not be suitable and alternative methods, like other types of electronic tagging, otolith microchemistry or genetics, should be used. A further concern with the use of PIT telemetry, or any other form of telemetry, is the effect of tagging and handling on the welfare of the fish. Consequently, the effect of tagging and handling on fish growth and survival has been well documented in scientific studies (Acolas et al., 2007; Jepsen et al., 2015; Larsen et al., 2013) and has resulted in suggestions for thresholds of minimum length for different sizes of PIT tags to reduce possible negative effects. For example, Acolas et al. (2007) suggested that 11.5 mm PIT tags could be used on brown trout with a minimum size of 5.7 cm (fork length) and Larsen et al. (2013) found no adverse effect of tagging on growth and survival of juvenile Atlantic salmon above 10 cm, tagged with 23 mm PIT tags. A recent meta-analysis, investigating the effect of tagging on juvenile salmonids has suggested a minimum length of 7 and 13 cm for fish tagged with 12 and 23 mm tags, respectively (Vollset et al., 2020). The threshold length for tagging juvenile brown trout with 23 mm PIT tags in this thesis was 11 cm, which is above the size limit suggested by Larsen et al. (2013). Although this length threshold was lower than that suggested by Vollset et al. (2020), it is within the size range, where the mortality from tagging and handling is still estimated to be low (less than 10%; Vollset et al., 2020).

2.4.2 Otolith microchemistry

Otoliths are small structures in the head of teleost fish, used for balancing and hearing (Popper et al., 2005). They are made of calcium carbonate (CaCO_3), organic matrix and a small quantity of other elements (Campana, 1999; Hüseyin et al., 2021). Otoliths already form at the embryonic stage and grow throughout the life of an individual (Campana, 1999). Due to differences in the ratio of formation between calcium and organic matrix, periodic (daily, seasonal) increments form, which provide an opportunity to infer individual's age and growth information (Morales-Nin, 2000). In addition, a range of elements get incorporated into the otoliths, which can be used to reconstruct migratory patterns or habitat utilization of individuals at specific life stages (Engstedt et al., 2010; Heidemann et al., 2012; Matetski et al., 2022). The success of using otolith microchemistry to discriminate between habitats is dependent on the extent to which the chemical composition of otoliths differs between habitats

(Avigliano, 2022; Turcotte & Shrimpton, 2020), as various factors, including the physio-chemical properties of water, ontogeny and physiology are known to have an effect on the integration of chemicals in the otoliths (Brown & Severin, 2009; Sturrock et al., 2015; Walther et al., 2010).

In **MS IV** the applicability of otolith microchemistry to identify strayers was tested among mature adult sea trout that had returned to freshwater to spawn. To achieve this, we obtained otoliths from juvenile fish from each river to form distinct baseline otolith fingerprints for each of the four rivers investigated in this thesis. These fingerprints were then used to build a Random Forest (RF) classifier to assign adult sea trout, captured during the spawning season, back to their natal rivers. In Mariager fjord, it was determined that otolith microchemistry works well at discriminating between juvenile brown trout originating from different rivers (**MS IV**). The overall classification accuracy for assigning juvenile trout to their natal rivers was 80%, which is considered a high accuracy, especially given the short spatial distances between the rivers (4-16 km). The classification accuracy however varied between the rivers, ranging between 66 and 95%. This is in agreement with the results from previous studies where high overall classification accuracies were obtained, but where variation in accuracy between nearby rivers was also documented (Matetski et al., 2022; Mikheev et al., 2021).

The discriminatory power of otolith microchemistry can be enhanced by including multiple elements into the assignment analysis. In **MS IV** no element had the ability to singlehandedly discriminate between the rivers, highlighting the importance of a multi-elemental approach. Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were determined to be the most important elements to be included in the RF classifier to achieve the highest discriminatory power. While these elements have been shown to provide high discriminatory power in other systems (Heidemann et al., 2012; Mikheev et al., 2021), other elements, like Ba/Ca, have been documented to provide high discriminatory power in other geographical areas (Nazir & Khan, 2019), despite being less important in discriminating between the rivers in Mariager fjord. **MS IV** also highlights that not all elements positively contribute to discriminating between individuals from different rivers, as Pb and Zn, which are commonly used in discrimination studies (Maguffee et al., 2019; Mikheev et al., 2021), were removed from the analysis as they did not improve discriminatory power of the RF classifier. **MS IV** demonstrated that otolith microchemistry could identify strayers in Mariager fjord system and could therefore be used in studies investigating patterns of straying. However, it is unclear whether otolith microchemistry would be able to detect fine scale patterns between multiple rivers, similar to the ones documented with PIT telemetry. More specifically, we documented in **MS I, II and III** that sea trout

may migrate between multiple rivers within the same spawning season or over multiple years, however as only the freshwater phase of the otolith was analysed for adult sea trout in **MS IV**, it was not possible to infer information about migratory patterns between multiple rivers during the same or multiple spawning season(s). Regardless, as PIT telemetry is not feasible in all systems, otolith microchemistry might still provide an alternative method to determine the overall prevalence of straying in a given system and how it relates to fish characteristics (e.g. sex and length).

The decision to use otolith microchemistry should be handled with caution, as it requires for individuals included in the study to be euthanized. Therefore, even in systems, where otolith microchemistry might be suitable to study population connectivity, the ethics of the use of otolith microchemistry should be considered, especially among vulnerable populations. In Mariager fjord, sea trout populations are in good condition (Birnie-Gauvin et al., 2018, Mikkelsen & Carøe, 2017) and no long-term negative effects are expected from the use of lethal sampling.

2.4.3 Genetics

The use of genetics has provided invaluable insights into the prevalence of reproductively successful straying in different salmonid species and populations (Bradbury et al., 2014; King et al., 2016; Ozerov et al., 2017). Genetics has mainly been used to qualitatively assess straying (Bekkevold et al., 2020; Massa-Gallucci et al., 2010), with quantitative estimates being scarce (Masson et al., 2018). Within the framework of this thesis, genetic analysis have provided insights into the implications of straying on the genetic structure of sea trout in four Mariager fjord rivers. Specifically, genetics revealed that all the individuals in this system likely form one population, instead of four genetically distinct populations. These are important findings, as it documents that strayers in Mariager fjord successfully spawn in non-natal rivers and suggests that straying likely also affects other population parameters, for example recruitment.

The genetic methods used in **MS IV** cannot, however, be used to determine how many of the strayers successfully spawn, since only a small number of individuals over several generations is required to successfully spawn in non-natal rivers for the genetic differences between individuals originating from different rivers to diminish (Waples & Gaggiotti, 2006). Consequently, the genetic approach used in **MS IV** cannot be used to evaluate straying in a quantitative manner, nor can it be used to infer information

about fine scale patterns of straying. It however cannot be excluded that alternative genetic methods, for example the use of a larger number of SNP markers, could provide further insights into possible genetic sub-structuring between rivers. Further, as genetic sampling is non-lethal it can be applied to all populations, even those in a vulnerable state.

2.5 Implications of straying

Straying to non-natal rivers may have significant implications for population dynamics and demographics of both the donor and recipient population(s) (Bett et al., 2017; Bowler & Benton, 2005). Strayers that successfully spawn in non-natal rivers directly contribute to the recruitment of the non-natal river, which affect the genetic structure and population dynamics of the given population (Jonsson et al., 2003; King et al., 2016; Massa-Gallucci et al., 2010). While the full implications of straying are dependent on the spawning success of strayers in non-natal rivers, straying likely has implications even when spawning results in failure. Strayers that have returned to a non-natal rivers to spawn likely compete with natal homers for spawning habitat and mates, and further represent a proportion of genetic information, potential breeders and their off-spring that will not be realized neither in their natal or non-natal river if the attempt to spawn results in failure.

In Mariager fjord, there are several indications that strayers likely regularly attempt to spawn in non-natal rivers, with at least a proportion of them being successful. More precisely, in **MS IV**, it was documented that strayers that are in non-natal rivers during the spawning season are mature and that there was temporally stable gene flow between the rivers, demonstrating that at least a proportion of strayers in this system successfully spawn in non-natal rivers. This supports the assumption of **MS I, II** and **III**, where it was hypothesized that the strayers, that were detected in a non-natal river during the spawning season - which was the majority of them - were there to spawn. Straying was also documented to be prevalent among repeat spawners (**MS II**), thus emphasizing the possible implications of straying to the population dynamics within the river, given the importance of repeat spawners for recruitment (Serbezov et al., 2012; Stubberud et al., 2022). Consequently, besides having an effect on the genetic structure among the rivers, strayers in Mariager fjord likely affect various population parameters, including recruitment.

The extent of the impact of straying further depend on the direction and intensity of straying between the recipient and donor population (Bett et al., 2017). For example, a large donor population that proportionally produces a low number of strayers, may still have a disproportionally high impact on rivers with smaller spawning population, given that the absolute number of individuals straying into such populations may be high compared to the size of the spawning population in the recipient river. There was some indication for such patterns in Mariager fjord. More precisely, in this thesis we documented that straying predominantly occurred towards the larger rivers, Villestrup and Kastbjerg, with only a small percentage of strayers entering rivers Valsgaard and Maren Møllebæk, possibly indicating reduced impact of straying in those rivers. However, when sampling the spawning populations in all the rivers, the proportion of strayers and natal-homers across rivers was similar (**MS IV**). Therefore, while smaller number of strayers return to Valsgaard and Maren Møllebæk, the absolute number of strayers in all the rivers is still somewhat proportional to the size of each spawning population, indicating that the *intensity* of straying is similar in all the rivers in Mariager fjord.

The results of this thesis have important implications that managers should consider when making decisions about sea trout populations in Mariager fjord, as well as in other systems. Assessing and understanding biological boundaries of populations is fundamental in making informed and effective management decisions, as it allows to identify possible stressors and bottlenecks of survival that impact population sustainability. In salmonids, population boundaries are often defined by the borders of the rivers or tributaries that individuals originate from or return to, which may not always be the case, as documented in this thesis. Additionally, in Mariager fjord it was documented that a proportion of sea trout migrate to multiple rivers throughout their life, making them subject to stressors from various habitats, which may consequently affect individual's survival and fitness. It further cannot be excluded, that the sea trout in Mariager fjord conforms to a meta-population structure, in which case the sustainability of sea trout in one river may therefore be dependent on the survival of sea trout in another river. Thus securing good habitat quality in all possible rivers individuals may migrate to could be essential in securing sustainability of sea trout populations in the wider area.

Conclusions

This thesis has significantly contributed to our understanding of straying behaviour among sea trout by providing novel insights into the prevalence of straying, its connection to various life-history patterns and by documenting various migratory patterns strayers undertake throughout their life. To study patterns of straying in Mariager fjord Denmark, multiple different methods were used, which included PIT telemetry, otolith microchemistry and genetics. While each method has its own set of advantages and disadvantages, all of them had the ability to provide complimentary information about various aspects of straying.

The occurrence of straying in salmonids is generally acknowledged, yet it is often overlooked and considered to apply to only a minority of the population, especially among wild salmonids. This thesis, however, documented that straying may be highly prevalent in sea trout populations, with an average of 37% of sea trout returning to freshwater in Mariager fjord documented to stray. Further, strayers in Mariager fjord displayed high variability in their migratory patterns depending on whether they had returned to freshwater for the first or several times. The majority of strayers that returned to freshwater for the first time were documented in a non-natal river only, while the majority of repeat spawning strayers were both detected in their natal and a non-natal river, with the migratory patterns changing from one year to the next. Albeit there were no differences in straying rates depending on the river of origin, straying towards the two larger rivers Villestrup and Kastbjerg was more common. The genetic results further indicate that reproductively successful straying in this system was confined to the fjord, since there was limited gene flow between the populations within and outside of the fjord.

Straying seems to be an inherent part of life-history of sea trout in Mariager fjord, as it was documented to be connected to multiple life-history characteristics at different life stages (juvenile and adult). We documented straying in both maiden and veteran spawners, in both small and large fish, in both spring and autumn migrants, in parr, pre-smolt, and smolts, and in individuals originating from all rivers. There was further indication that the aforementioned patterns of straying could be linked to individual fitness, however the underlying causes for why some individuals stray and others do not remain unclear and would benefit from further research.

The prevalence of straying and the patterns it is associated with has had implication for the genetic structure and likely to other population parameters, like demographics and recruitment. The genetic results indicate that strayers in Mariager fjord spawn in non-natal rivers, which has led to the loss of

genetic structuring between the rivers, meaning sea trout in Mariager fjord, independent of their river of origin, form one population. However, the extent of successful spawning and fitness related costs associated with returning to a non-natal river instead of the natal river are still unclear and would require further research to clarify the full extent of implications of straying in this system. Nevertheless, the results of this thesis have management implications, as sea trout were documented to migrate to multiple rivers over the course of their life, and are thus subject to stressors of multiple habitats, indicating towards the need to secure good habitat quality in all possible habitats sea trout may migrate. Based on the results of this thesis it is safe to conclude that sea trout is a magnificent (and challenging) species to study, that displays high variability in its life-history tactics across life stages. While several novel and important insights into the life-history of strayers were obtained throughout this thesis, there are various questions that still persists and could provide avenue for future research. Of those, determining the fitness related costs of straying, exploring the levels of straying in other geographical areas, and resolving the underlying mechanisms that cause straying should be at the forefront of future work on this topic. My sincere hope is that this thesis has provided inspiration to salmonid researchers, who would be encouraged to carry on the work to fill these knowledge gaps and further advance our understanding of strayers, sea trout and salmonids.

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High prevalence of straying in a wild brown trout (*Salmo trutta*) population in a fjord system

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Natal homing is a prevalent life-history strategy among salmonids. However, not all individuals return to their natal river, a behaviour known as straying. In this study, we investigated the prevalence of straying and its connection to different life-history characteristics in an anadromous brown trout (*Salmo trutta*) population. In total, 21 538 juvenile out-migrating brown trout were tagged with Passive Integrated Transponder (PIT) tags in two years. Individuals were grouped according to their developmental status (parr, pre-smolt, and smolt) at the time of out-migration to investigate the effect of such life-history characteristic on the likelihood of straying. High number of anadromous brown trout (36%) were detected in non-natal rivers. Individuals spending longer time at sea were less likely to stray. Additionally, the likelihood of straying was dependent on the developmental status during out-migration, with parr having lower likelihood to stray compared to pre-smolt and smolt. However, the latter is further dependent on length and timing of juvenile out-migration. These results indicate that straying is an inherent part of this anadromous brown trout population and it is influenced by several life-history characteristics at different life stages. This may have significant implications to genetic structure within and between populations and to population dynamics.

Keywords: dispersal, natal homing, salmonids, sea trout, straying.

Introduction

Natal homing is a common life-history strategy among freshwater, marine, and anadromous fishes (Svedäng *et al.*, 2007; Engstedt *et al.*, 2014; Chen *et al.*, 2020). It is an evolutionary strategy in which case individuals return to spawn in the same area where they had hatched. From an evolutionary sense, its main advantage is to increase individual fitness through increased probability of finding suitable breeding grounds and partners during the breeding season (Quinn, 1993; Keefer and Caudill, 2014). It is also a mechanism through which adaptations for local environmental conditions develop, which further increases individual fitness (Quinn, 1993; Hendry *et al.*, 2004).

Salmonids are well known for their fidelity to their natal spawning grounds and their ability to home (Quinn, 1993; Klemetsen *et al.*, 2003). Thus, homing has become rooted into the description of salmonids' lifecycle. However, not all individuals return to their natal breeding grounds; a behavioural trait referred to as straying (Quinn, 1993; Degerman *et al.*, 2012; King *et al.*, 2016). Among taxa that display strong natal homing behaviour, straying is typically perceived as a failure, or maladaptive behaviour, but from an evolutionary perspective, it rather acts as a complimentary mechanism to homing, as it is an important mechanism in order to colonize or re-colonize suitable habitat (Quinn, 1993; Knutsen *et al.*, 2001; Massa-Gallucci *et al.*, 2010). Further, it provides gene-flow between populations and genetic variability within populations, which may buffer against environmental variability by strengthening a population's resistance to change.

Genetic studies of salmonid population connectivity have implied that straying is more common than the classic life-history theory for salmonids suggests. Numerous genetic stud-

ies have documented varying levels of gene flow between salmonid populations originating from different rivers (Ayllon *et al.*, 2006; Massa-Gallucci *et al.*, 2010), suggesting that at least a part of a population does not return to its natal river. The extent of straying may however vary significantly between different systems. For example, there is documentation that rivers in close proximity exhibit high genetic similarities or even form homogenous population that are genetically indistinguishable from each other (Østergaard *et al.*, 2003; Ayllon *et al.*, 2006; Schtickzelle and Quinn, 2007). This means there must be individuals regularly migrating to non-natal rivers and successfully spawning there. However, this is not the case for all systems, as many studies have found distinct genetic structuring among near-by populations or even within the same watershed, indicating low levels of gene-flow (Hindar *et al.*, 1991; Primmer *et al.*, 2006; Vähä *et al.*, 2007), thus suggesting high fidelity towards their natal spawning areas. Although differences in methodologies between the aforementioned studies may account for some of the observed differences in the results, there are also studies from proximate rivers using the same methodology that produced different levels of genetic differentiation (Massa-Gallucci *et al.*, 2010; Bekkevold *et al.*, 2020).

Even though genetic methods have provided valuable insights into the prevalence of straying among salmonids, quantitative estimates for straying rates on a population level, especially among wild fish, are limited. Most quantitative studies among salmonids have focused on straying rates in stocked fish (Heggberget *et al.*, 1991; Jonsson *et al.*, 2003) which have been documented to stray to a higher degree compared to wild fish (Jonsson *et al.*, 2003; Jonsson and Jonsson, 2014). It has been proposed that stocked fish may have missed some

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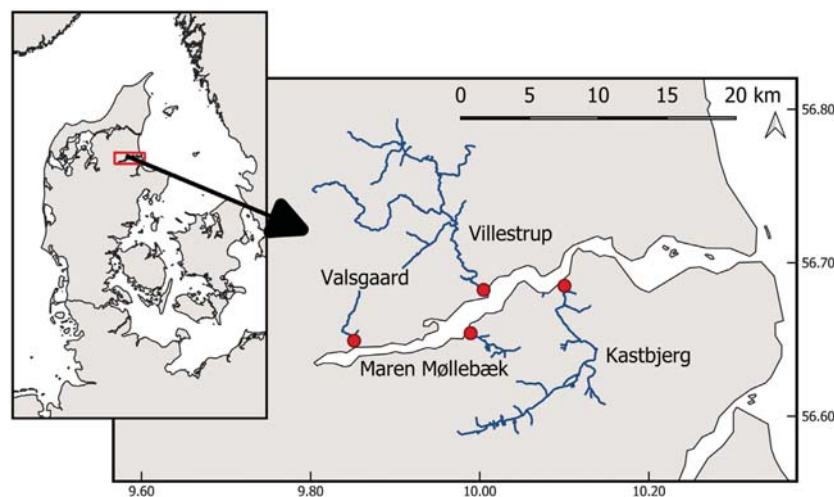


Figure 1. Rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk flow into Mariager Fjord, Denmark. Juvenile out-migrating sea trout were PIT-tagged in river Villestrup in 2015–2016. Their movements in and out of their natal river, as well as rivers Kastbjerg, Valsgaard, and Maren Møllebæk were monitored using PIT antennas (identified by red dots on the map).

crucial imprinting steps as juveniles in their natal river, which may have impaired their natal homing abilities (Keefer and Caudill, 2014). However, given that straying is also common among wild populations, a variety of factors, other than origin (i.e. stocked vs wild), may effect straying in salmonids, including environmental stability (Quinn and Tallman, 1987; Østergaard *et al.*, 2003) and quality (Moore *et al.*, 2007).

In this study straying behaviour of anadromous brown trout (*Salmo trutta*), also referred to as sea trout, was investigated in order to (i) quantify the prevalence of straying in a sea trout populations, and (ii) to determine how different life-history characteristics affect individual likelihood to stray. To investigate these questions, juvenile out-migrating brown trout were tagged in two years with Passive Integrated Transponder (PIT) tags at the outlet of a lowland river during juvenile out-migration in spring. Their return to their natal river and three additional adjacent rivers were monitored by PIT-antennas at the mouth of all the rivers. In order to investigate the effect of life-history characteristics on straying rates all fish were grouped at tagging according to their developmental status as parr, pre-smolt, and smolt.

Methods

Study sites

Mariager Fjord is situated on the east coast of Jutland in Denmark and connects to the Kattegat through a narrow opening to the east. The fjord is about 40 km long, 2 km wide and has a maximum depth of 30 m. The surface water salinity varies from around 12 psu in the inner part of the fjord to >20 psu in the outer part of the fjord (Fallesen *et al.*, 2000).

River Villestrup, the largest river discharging into Mariager Fjord, is located on the north coast of the fjord. The river is about 20 km long and has a catchment area of 126 km². The mean annual discharge of the river is 1.1 m³s⁻¹ and the flow is stable due to large groundwater inflow (Olesen, 2011). River Kastbjerg is the second largest river flowing into the fjord and is located on the southern side of it. It has a length of 18 km and a catchment area of 99 km². Two brooks, Valsgaard and Maren Møllebæk, are roughly 5 km in length and also discharge into the fjord (Figure 1).

Tagging and tracking of the individuals

Out-migrating juvenile brown trout were caught in a Wolf-type trap (Wolf, 1951) in the River Villestrup in the spring of 2015 and 2016. The trap was located 300 m from the mouth of the river and it was designed to cover the full width of the river in order to catch all descending trout >10 cm. All trout >11 cm were tagged and grouped according to their developmental status as parr, pre-smolt, or smolt (according to Tanguy *et al.*, 1994). The main morphological characteristics used to assign fish to aforementioned groups were the presence/absence of parr marks, coloration of the body and fins, and the shape of the body. The trap was operating over the course of the entire spring out-migration of brown trout from mid-/late-March until the beginning of June in both years. All fish were anaesthetized with benzocaine (25 mg/l, Sigma Chemical Co., St Louis, USA), then measured (total length, to the nearest mm). Fish were PIT tagged with 23 mm tags (Texas Instruments, RI-TRP-RRHP, half duplex, 134 kHz, length 23.1 mm, diameter 3.85 mm, and weight 0.6 g in air) by experienced field technicians via a small incision ventrolaterally, posterior to the pectoral fin. After tagging, fish were transferred to a recovery tank and subsequently released downstream of the trap, 200 m upstream from the outlet. The methods, trapping, handling, and PIT tagging, used within the scope of this study are common and documented to have a minimum effect on the tagged fish (Acolas *et al.*, 2007; Jepsen *et al.*, 2008; Larsen *et al.*, 2013). All protocols used in this study were approved by the Danish Experimental Animal Committee (2017–15–0201–01164).

In order to register all possible up- and down-stream movements of the tagged individuals in river Villestrup, Kastbjerg, Valsgaard, and Maren Møllebæk, PIT-antenna systems were mounted a maximum of 400 m upstream from the outlet of each of the rivers. Each system consisted of two antennas, approximately 10 m apart, so that direction of movement could be inferred from the sequence of detections. The systems continuously registered the date, time and individual specific code of tagged fish that passed through them. PIT-antennas operated continuously from March 2015 until February 2020. The last data download from the system was done 29 February 2020.

Table 1. Total number of fish tagged in different developmental groups, the number of individuals returning to freshwater (natal homer or strayer) divided by year of tagging and the total length (\pm standard deviation) of the tagged individuals pooled across years.

Group	Tagged*	Natal homer **	Strayer**	Tagged*	Natal homer**	Strayer**	Total length (\pm SD)
		2015			2016		
Parr	1199 (7.3%)	90 (76.9%)	27 (23.1%)	469 (9.1%)	22 (59.5%)	15 (40.5%)	138.8 (\pm 16.3)
Pre-smolt	6830 (41.7%)	410 (65.4%)	217 (34.6%)	3426 (66.6%)	121 (52.8%)	108 (47.2%)	141.1 (\pm 16.4)
Smolt	8365 (51.0%)	492 (65.6%)	258 (34.4%)	1249 (24.3%)	37 (49.3%)	38 (50.7%)	150.3 (\pm 19.1)
Total	16 394	992 (66.4%)	502 (33.6%)	5144	180 (52.8%)	161 (47.2%)	145.0 (\pm 18.3)

* Percentage is calculated based on the total number of individuals that were tagged in a given year.

** Percentage is calculated based on the total number of individuals that were detected returning to freshwater among individuals tagged in a given developmental group and year.

Data processing and statistical analysis

In this study, individuals that were detected in non-natal rivers (Kastbjerg, Valsgaard, and Maren Møllebæk) were referred to as strayers. In addition, to explore whether straying events were linked to possible spawning events, a spawning season was defined to last from 15 November until 31 December. Therefore, all individuals that had entered a river prior to 15 November, without being detected leaving before that date or were detected entering a river between 15 November to 31 December were considered to be in the river with the aim to spawn. It is possible, depending on environmental conditions that sea trout spawn outside of that window as well, but to our knowledge, the majority of spawning takes place within that window of time (Rasmussen and Pedersen, 2018).

The present study focused on the movements of sea trout during their first year of return to freshwater after leaving as juveniles. A year is defined to last from 1 May until 30 April the following year. The majority of the individuals were only detected during one year, but 206 individuals were detected over multiple years. The subsequent movements of these individuals over additional years is not covered within the scope of this study. During data processing, all detections within 30 days post-tagging were excluded from the dataset, as it was not possible to conclude whether these individuals had left the river within that time, as the fish were released approximately 300 m from the outlet of the river. Further, individuals, which did not follow the expected river-sea-river migratory pattern, meaning their migration between the river and the sea had been partially missed by the antennas, were removed from the dataset. This resulted in total 178 fish being removed from the dataset.

Overall return rate was calculated based on the percentage of all tagged individuals that were detected at any of the PIT -antennas in the four rivers. In addition, return rate to river Villestrup (i.e. homing) was calculated based on the percentage of all tagged individuals that were detected by the antennas in river Villestrup. Time spent at sea was calculated based on the number of days that had passed between tagging of the individual and its first detection at any of the PIT -antennas in the fjord.

Data were analysed using Bernoulli distributed Generalized Linear model (GLM) with a logit-link function in order to investigate the effect of fish length (L_T), day of out-migration (DM), developmental status (DS), days at sea (DaS) and year of tagging (Y) on individual likelihood of straying (S). Original model included the main effect of all the previously named variables and all possible two-way interactions. Parsimonious model selection was done using stepwise backwards model selection based on AIC values (Zuur *et al.*, 2009).

Table 2. Number of fish that returned to their natal river (natal homers) and strayed (strayers) (with percentage in bracket), depending on which river(s) they were detected in.

Migration type	No. fish (%)
Natal homers (Villestrup)	1172 (63.9%)*
Strayers:	663 (36.1%)*
Kastbjerg	455 (68.6%)**
Villestrup and Kastbjerg	156 (23.5%)**
Valsgaard	27 (4.1%)**
Villestrup and Valsgaard	8 (1.2%)**
Kastbjerg and Valsgaard	7 (1.1%)**
Maren Møllebæk	5 (<1%)**
Villestrup, Kastbjerg, and Valsgaard	4 (<1%)**
Maren Møllebæk and Kastbjerg	1 (<1%)**

*Percentage is calculated based on the total number of sea trout that returned.

**Percentage is calculated based on the total number of sea trout that have strayed.

The final model fitted to the data is as follows:

$$S_{-ij} \sim \text{Bern}(\pi_{ij})$$

$$\text{Logit}(S_{-ij}) \sim L_T + \text{DM} + \text{DS} + \text{DaS} + Y +$$

$$L_T : \text{DS} +$$

$$\text{DS} : \text{DM} +$$

$$\text{DaS} : \text{DM} +$$

$$\text{MC} : \text{DM}$$

Data was analysed with R (v 4.0.5).

Results

Return rate and rate of straying

In total, 21 538 sea trout were tagged in 2015 and 2016, of which 7.74% were parr, 47.62% pre-smolt, and 44.64% smolt (Table 1). Out of all tagged individuals 1835 (8.5%) sea trout were registered returning either to their natal river or nearby other river(s) included in this study. Of all returning fish, 663 (36%) were detected in non-natal rivers Kastbjerg, Valsgaard or Maren Møllebæk during the first season in which they returned to freshwater (herein referred to as strayers, Table 2). The majority of the strayers were detected in river Kastbjerg (69%) only, or in both river Kastbjerg and their natal river (24%). A few individuals were detected in rivers Valsgaard and Maren Møllebæk, or in multiple non-natal and natal rivers (Table 2).

Yearly return rates were 9.1% and 6.6% for fish tagged in 2015 and 2016, respectively (Table 3). The return rate

Table 3. Number of natal homers and strayers among returned fish that were tagged in 2015 and 2016. Percentages are calculated based on the total number of fish that had returned to freshwater, divided by the year of tagging.

Year	2015	2016
Natal homers	992 (66.4%)	180 (52.8%)
Strayer	502 (33.6%)	161 (47.2%)
Only non-natal river	360 (71.7%)*	135 (83.9%)*
Natal & non-natal rivers	142 (28.3%)*	26 (16.1%)*
Total number of returners	1494	341

*percentage is calculated based on the total number of strayers that had returned to freshwater, divided by the year of tagging.

exclusively to river Villestrup (excluding fish that were only detected in non-natal rivers) was 6.2% on average, more specifically 6.9% and 4.0% among fish tagged in 2015 and 2016, respectively.

Migration timing

We observed two waves in the timing of return: the first wave lasted from approximately June until September, while the second wave lasted from early October until mid-January (Figure 2). Of all the sea trout that were only detected in non-natal rivers, the majority of them (80%) had entered it between June and December. Further, the majority of them were not detected leaving the river before the beginning of the spawning season, suggesting that these strayers were likely in non-natal rivers during the spawning season (Table 4).

A smaller group of individuals were detected in both their natal and non-natal river (Table 4), mostly in the non-natal river prior to migrating to their natal river. Further, most of the previously named individuals (66%) had migrated out of a non-natal river before the spawning season, with majority (73%) doing that between October and mid-November. Almost all of the individuals (98%) that migrated out of the non-natal river prior to the spawning seasons were subsequently detected entering their natal river but not leaving before the beginning of the spawning season. A small fraction of individuals ($n = 51$) were detected migrating out of a non-natal river during the spawning season, and most of them ($n = 39$) subsequently entered their natal river. There were a few individuals that had been to their natal river prior to migrating to a non-natal river.

Individual likelihood of straying

The summary statistics of the Generalized Linear Model (GLM) outcome are presented in Table 5. The likelihood of straying decreased with increasing time individuals spent at sea (GLM: Days at sea, $F = 18.5$, $df = 1$, $p < 0.001$, Figure 3), which was also dependent on the timing of out-migration as juveniles. Juveniles that migrated out earlier in the season and stayed out the longest during their marine phase, had the lowest likelihood of straying (GLM: Days at sea \times day of out-migration; $F = 7.4$; $df = 1$, $p = 0.006$, Figure S1). The differences between the likelihood of becoming a strayer and previously named variables are apparent among individuals that have spent the longest time away at sea. Given that the effect of the interaction is most likely driven by the small number of individuals that spent the most amount of time at sea (75% of fish had returned within 572 days, which is also the point where differences become apparent, see Figure S1), and

the overall pattern between the likelihood of straying against time spent at sea between earlier and later out-migrants is similar, this interaction will not be further discussed in the present study, as it does not significantly contribute to the overall ecological relevance of the results.

The model also suggests a relationship between the rate of straying and the developmental status: parr had lower likelihood to stray compared to pre-smolts and smolts (GLM: Developmental status; $F = 17.9$; $df = 2$; $p = 0.01$; Table 1). However, this relationship was dependent on the timing of out-migration (GLM: Developmental status \times day of out-migration; $F = 16.3$, $df = 2$, $p < 0.001$, Figure 4.) and length at tagging (GLM: Developmental status \times Length, $F = 7.5$; $df = 2$, $p = 0.02$, Figure 4), independently. There was a negative relationship between the timing of migration and the likelihood of straying for parr and pre-smolt, which is more evident for the parr group. In contrast, a positive relationship was documented in the smolt group: the later in the season they migrate the more likely they are to stray. Similarly to previous, there was an inverse relationship between total length as juveniles and likelihood of straying among parr and pre-smolt group, though considerably more evident in the former. However, there seemed to be no effect of length on the likelihood of straying in the smolt group.

Further, according to the model, the likelihood of straying was not uniform across years (GLM: Year, $F = 20.0$; $df = 1$; $p < 0.001$). Fish tagged in 2016 strayed to a higher degree compared to fish tagged in 2015 (Table 3).

Discussion

The prevalence of straying

The results of this study demonstrate high rates of straying to close-by rivers for individuals originating from one donor population. Further, the likelihood of straying was associated with several life-history characteristics. Straying in general acts as an important evolutionary mechanism through which a variety of factors, for example population dynamics and the genetic structure of both donor and recipient population, are affected (Keefer and Caudill, 2014; Bekkevold *et al.*, 2020). Therefore, the results presented in this study may have important evolutionary implications, which are crucial to take into account when making management decisions about such populations.

In total, 36% of the returned sea trout were detected in a non-natal river and are subsequently referred to as strayers in the present study. The fact that sea trout stray to non-natal rivers is not surprising, as straying has been documented in several systems using genetic markers (Østergaard *et al.*, 2003; Massa-Gallucci *et al.*, 2010; King *et al.*, 2016). However, quantitative estimations of straying rates at the population level among sea trout are limited, especially in wild populations, not impacted by stocking. Using a range of approaches, different studies have found straying rates in wild sea trout populations 1.6% to 15% (Berg and Berg, 1987; Jonsson and Jonsson, 2014), which are significantly lower estimates than those documented in the present study.

The straying rates documented in this study were likely minimum estimates. Even though there are several different methods available to study straying behavior in migratory fish, including telemetry, genetics, and otolith microchemistry

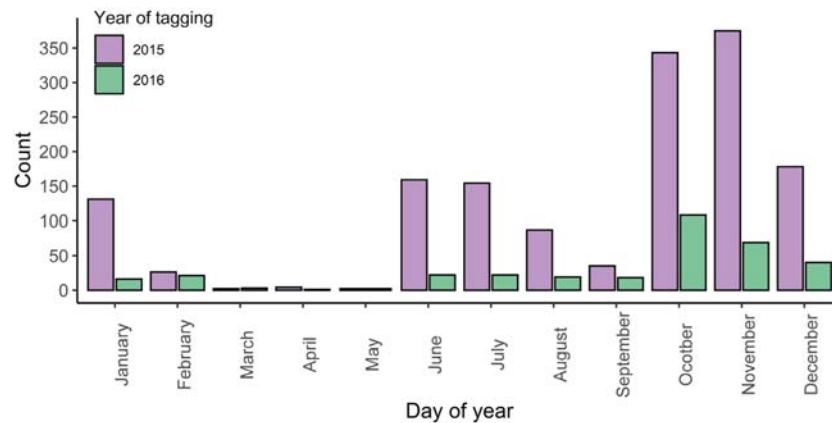


Figure 2. Number of fish returning for the first time and timing of their entry to rivers Villestrup, Kastbjerg, or Valsgaard relative to the month. Different colors represent individuals tagged in different years.

Table 4. Migration pattern and timing among individuals that were either only detected in non-natal river(s) or both natal and non-natal river(s).

Group	No. fish detected	No. fish entering a non-natal river between 1 June–31 December	No. fish leaving non-natal river before spawning season	No. of fish detected within natal river during spawning season
Non-Natal	495	398	52	–
Natal & non-natal	168	162	107	155

Table 5. Output of most parsimonious GLM model with bold entries representing variables where $p < 0.05$.

Variable	df	Deviance	<i>p</i> -value
Length	1	2.1	0.15
Days at sea	1	18.5	<0.001
Developmental status	2	17.9	0.01
Year of tagging	1	20.0	<0.001
Day of outmigration	1	1.8	0.2
Length × Developmental status	2	7.5	0.02
Days at sea × day of out-migration	1	7.4	0.006
Developmental status × Day of out-migration	2	16.3	<0.001
Developmental status × days at sea	2	5.6	0.06

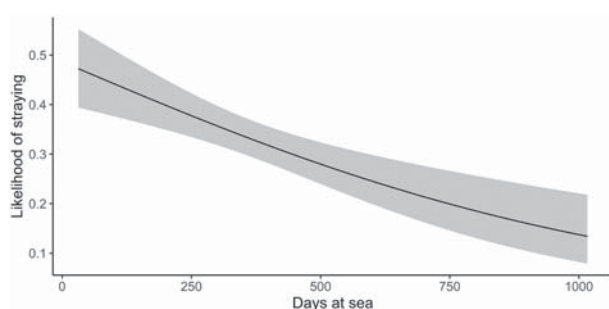


Figure 3. Modelled output of straying probability against time spent at sea (days). The model suggest the likelihood of straying is dependent on the duration of marine phase. The line and the shaded area represent the mean and the 95% confidence intervals of the model output. The plot is conditional of total length, fixed at mean TL = 146 mm; day of out-migration, fixed at DM = 110; developmental status and year of tagging, fixed at smolt and 2015, respectively.

(Jonsson *et al.*, 2003; Massa-Gallucci *et al.*, 2010; Martin *et al.*, 2013), all of them entail a common shortcoming: it is (almost) impossible to monitor all possible rivers that fish may enter to or originate from. This is most likely also the

case in this study. In river Villestrup, a divergence in migratory behavior of smolts has been documented: approximately half of them stay in the fjord for extended periods of time and the other half migrate out of the fjord to Kattegat relatively quickly after leaving the river (del Villar-Guerra *et al.*, 2014). As there are no PIT-antennas on the rivers outside of the fjord, it cannot be excluded that individuals that leave the fjord may enter them. Although, straying in salmonids has been most commonly documented in rivers close to their natal river (Knutset *et al.*, 2001; Jonsson *et al.*, 2003; Bekkevold *et al.*, 2020), there is also documentation from several systems where salmonids have been registered to enter rivers significantly further away (Degerman *et al.*, 2012; Martin *et al.*, 2013; Birnie-Gauvin *et al.*, 2019).

In addition, even though the most important sea trout rivers within the fjord were monitored, there are additional smaller brooks, where sea trout could have strayed, that were not. Thus, it cannot be excluded that some of the sea trout may have 1) visited other non-monitored rivers and subsequently returned to river Villestrup, or 2) have strayed to other non-monitored rivers and did not return to river Villestrup. Both of these options would further increase the proportion of strayers documented here. However, as river entry to smaller rivers,

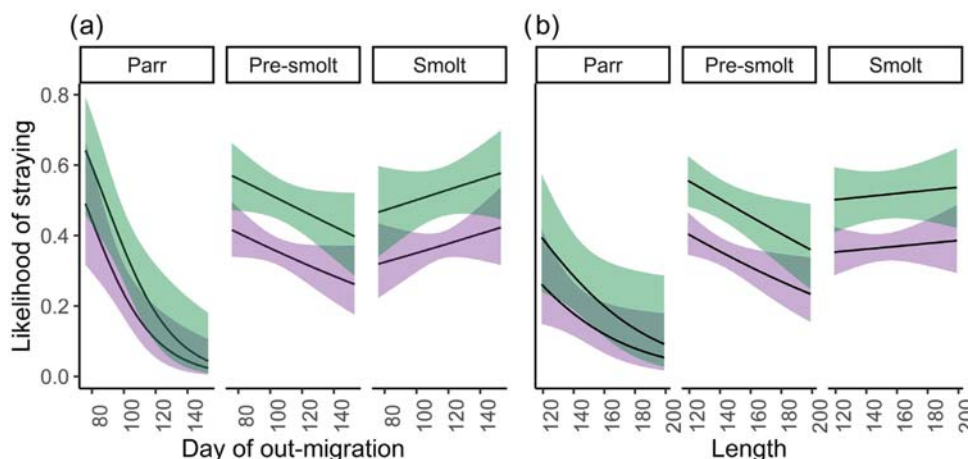


Figure 4. Modelled output of the likelihood of straying against (a) day of out-migration and (b) length at tagging among sea trout tagged in 2015 (purple) and 2016 (green). The lines and the shaded areas represent the mean and the 95% confidence intervals of the model output. The plot is conditional on (a) total length, fixed at mean LT = 146 and days spent at sea, fixed at DaS = 284; and (b) day of outmigration, fixed at DM = 110 and days at sea, fixed at mean DaS = 284.

such as Valsgaard and Maren Møllebæk, were rare, straying to other smaller brooks within the fjord is likely to be even rarer.

In total, 8% of the tagged individuals returned to any of the monitored rivers discharging into the fjord, while only 6% returned to the natal river. A common method to estimate marine survival in anadromous fish is to compare the number of juveniles leaving a river to the number of adults returning (Friedland, 1993; Koslow *et al.*, 2002), or to tag a part of the population and document their return (Jonsson and Jonsson, 2009; del Villar-Guerra *et al.*, 2019). Our results suggest that especially when using these common methods to obtain estimates on marine survival, the overall return rate of fish is likely to be underestimated, for example if only one river is monitored in tagging studies. Consequently, survival at sea will also be underestimated as more fish will have returned to freshwater, albeit to a non-natal river, than are accounted for. This consideration is particularly relevant for systems where there are multiple nearby rivers that present alternative habitats for fish to migrate. However, as the first method is also dependent on the number of fish straying *into* the river in question, the effects on the accuracy of marine survival estimates may not be as pronounced. Moreover, as the returned individuals may be of mixed origin this method may still not provide an accurate estimate the survival of individuals from the river in question.

To spawn or not to spawn in a non-natal river?

The majority of the strayers (69%) were solely detected in non-natal rivers, with a large fraction detected in the non-natal river during the spawning season. While it was not possible to observe spawning events, these findings may nonetheless suggest that these strayers were attempting to spawn, as all returning individuals were in the age class where sexual maturity can be reached (Klemetsen *et al.*, 2003), and thus may be considered true reproductive strayers. This would further suggest that there is high level of gene flow between different rivers (especially Kastbjerg and Villestrup), possibly implying that all or some of the rivers within the fjord make up a meta-population and for that matter may benefit from being managed as a sin-

gle unit (Schtickzelle and Quinn, 2007). However, in order for the rivers to conform to meta-population structure the success of this life-history pattern, in the form of fitness, must be evaluated. It cannot be excluded that spawning in non-natal rivers results in low reproductive success or in complete failure (Peterson *et al.*, 2014; Mobley *et al.*, 2019). Genetic assignment studies of offspring from all the rivers and/or genetic studies into population differences could provide important information in this realm.

It cannot be excluded that straying sea trout were immature during river entry and ascended to a non-natal river for overwintering (Thomsen *et al.*, 2007), which is a common migratory behaviour for sea trout at these latitudes (Degerman *et al.*, 2012; King *et al.*, 2016). However, we find it to be unlikely, as immature individuals have rarely been captured in the catches during electrofishing surveys of the spawning population, which have been conducted in river Villestrup over multiple years (Unpublished data). In addition, similar patterns (low number of adult immature sea trout entering rivers during winter) have also been documented in another Danish river that flows into a fjord system (Rasmussen and Pedersen, 2018), which may imply that lower salinity in the fjord does not cause such adverse physiological affects, which would require individuals to ascend to freshwater during winter.

A smaller group (approximately one third) of strayers were detected both in their natal and non-natal rivers. The majority of these fish visited a non-natal river prior to migrating to their natal river, and their movement from the non-natal to the natal river occurred shortly before or during the spawning season. The proximate causes for this kind of migratory pattern is unclear, however it cannot be excluded that this is a part of spawning migration as movement between different habitats prior to spawning has been documented among sea trout (Finstad *et al.*, 2005; Östergren *et al.*, 2011). This has been proposed as a strategy, through which individuals increase their reproductive success by breeding in multiple locations with multiple partners (Evans, 1994; Taggart *et al.*, 2001; Finlay *et al.*, 2020). This may also apply to the individuals tagged in this study, especially to those detected in a non-natal and natal river *within* the spawning season. Further, even though majority of the individuals that had been to

both natal and non-natal rivers migrated out of the non-natal river before the spawning season, they did so within the period when spawning may occur (October to mid-November) in these rivers. This means they possibly tried to hedge the risk of unsuccessful spawning by attempting to reproduce in multiple locations. Since all the rivers in this system are in close proximity to each other, sea trout could easily migrate from one river to another in a short amount of time. Further, competition for spawning habitat and mates is most likely high in their natal river, given that the population density is high (Birnie-Gauvin *et al.*, 2018). In this case, it may be more beneficial for individuals to return to non-natal river instead of their natal river to increase their reproductive success.

The effect of different life-history aspects on the likelihood for the individual to stray

In this study, it was documented that the rate of straying was dependent on the duration of the marine migration; the longer time the fish spent at sea, the less likely they were to stray. This was a somewhat surprising result, as even though there are examples for the opposite (Hard and Heard, 1999), the overall pattern seem to suggest that the longer fish are away at sea, the more likely they are to stray (Quinn, 1993; Jonsson *et al.*, 2003). It has been hypothesised that older fish may “forget” the route back to their natal river as the environmental cues used to recognize it have changed overtime, or the ability for individual to remember them may have reduced (Jonsson *et al.*, 2003). This does not seem to be the case for fish migrating from river Villestrup. It may instead be hypothesised, similarly to Hard and Heard (1999), that the differences documented in this study in the likelihood of straying between earlier and later returners, may be related to the differences in evolutionary strategies between the sexes. However, as the sex of the individuals tagged in this study was not determined, further research is required.

We further found that straying behaviour is associated with several life-history characteristics connected to the juvenile phase of life, primarily to the developmental status during out-migration. In average parr were less likely to stray compared to pre-smolts and smolts. This is surprising, as physiological processes occurring over the parr-smolt transformation, known as smoltification, have been linked to be essential in natal imprinting (Lema and Nevitt, 2004; Björnsson *et al.*, 2011). As a result, it has been suggested that individuals that have not gone through these essential steps have higher likelihood to stray (Keefer and Caudill, 2014). However, Armstrong *et al.* (2021) has documented among Atlantic salmon (*Salmo salar*) that changes in thyroid hormone levels, which are associated with natal imprinting (Lema and Nevitt, 2004), also occur at earlier life-stages, which implies that natal imprinting takes place over an extended period of time and not only over the smoltification process. This seems to be also supported by the findings of this study, as parr, which at least according to their appearance, had not gone through smoltification during sea entry, were able to locate their natal river and return to it to a higher extent than pre-smolts and smolts. In addition, this may further support our hypothesis that the primary cause for straying in this population is not an individual failure to recognize their natal river; it may instead be a part of a life-history strategy aiming to increase individual fitness.

The likelihood for parr, pre-smolt or smolt to stray is further dependent on the timing of juvenile out-migration from

their natal river and individual length during out-migration, independently. The effect of the aforementioned variables is the most pronounced in parr group and less in pre-smolt and smolt. The underlying mechanisms driving the differences between the groups are unclear. However, as smoltification and natal imprinting are complex behavioural and physiological processes that are deeply interconnected with each other (Boeuf *et al.*, 1989; Lema and Nevitt, 2004; Björnsson *et al.*, 2011), the patterns documented here are most likely influenced by multiple intrinsic or extrinsic factors. Additionally, in combination with our previous findings, these results suggest that the likelihood for the individuals to stray is part of a complex process, which is affected by several life-history characteristics at different life stages.

Conclusions

In this study, we documented that on a population level high number of sea trout strayed from one donor population to nearby rivers in a fjord system. Therefore, straying, either temporarily or for the purpose of reproduction, appears to be an inherent part of different life-history strategies this sea trout population displays. Quantitative estimates of straying rates in wild sea trout populations, are scarce so this study contributes greatly to the understanding of straying as an alternative life history strategy for the species. We have further documented that straying is connected to several life-history characteristics that have an effect on the individual likelihood to stray at different stages of life. However, as the potential implications of straying to population dynamics and genetic structure of both the donor and recipient population is outside the scope of this study, further research is needed to elucidate the consequences of straying in this system. Regardless of implications of straying, individuals that stray are exposed to the conditions of at least two habitats for short and/or long periods of time. These individuals may thus be exposed to different anthropogenic and/or environmental threats. Therefore, this study clearly illustrates the importance of securing good habitat quality in all possible areas sea trout may inhabit, which is particularly important to take into account by management and policy makers.

Supplementary material

Supplementary material is available at the ICESJMS online version of the manuscript.

Conflict of interest

Authors have no conflict of interest to declare.

Author contribution

All authors contributed to the conception and design of the study. KK and HB significantly contributed to analysing the data. All authors contributed significantly to the interpretations of the results. All authors have given their final approval for the manuscript to be published.

Data availability

Data can be provided by the authors upon reasonable request.

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Supplementary material: “High prevalence of straying in a wild brown trout (*Salmo trutta*) population in a fjord system”

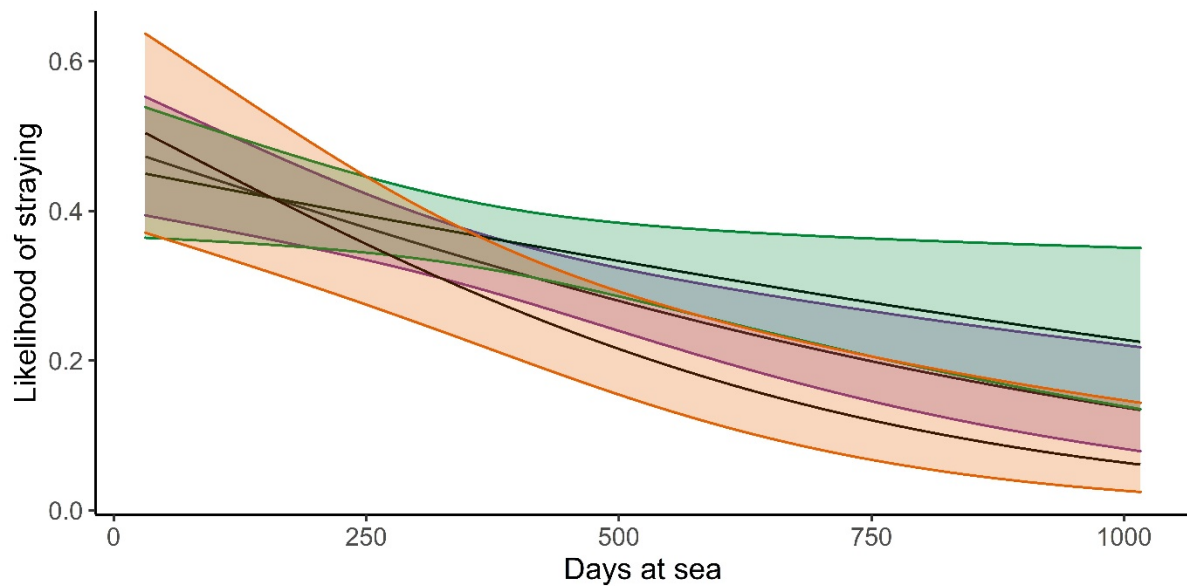


Figure S1. Modelled output of straying probability against time spent at sea (days). The model suggests that the likelihood of straying is dependent on the duration of marine phase and the timing of out-migration as juveniles. The lines represent the 10% (orange), 50% (purple) and 90% (green) quantile of day of out-migration, representing juveniles that migrated early, in the middle or late in the season. The shaded areas represent the 95% confidence intervals of the model output. The plot is conditional of total length, fixed at mean TL=146 mm; morphological group and year of tagging, fixed at smolt and 2015, respectively.

MANUSCRIPT II: Variability in straying behaviour among repeat spawning anadromous brown trout (*Salmo trutta*) followed over several years

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Variability in straying behaviour among repeat spawning anadromous brown trout (*Salmo trutta*) followed over several years

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Natal homing, a strategy in which individuals return to breed in the same river as they were born, is prevalent among salmonids. However, some individuals may not return to their natal river, and instead stray to non-natal rivers. To date, there is limited documentation on patterns of straying among iteroparous salmonids that have returned to spawn over multiple years. In this study, 21538 out-migrating juvenile anadromous brown trout (*Salmo trutta* L.) were tagged over two years with passive integrated transponder (PIT) tags, and followed as they returned to either their natal or three non-natal rivers in a fjord system, over multiple years. In total, 206 individuals were detected returning for more than one year, of which 43% were strayers. A divergence in migratory strategies among strayers was documented: a smaller proportion of strayers were only detected in non-natal rivers, while the majority (67%) displayed high variability in their migratory patterns as they were detected in both their natal and non-natal rivers, sometimes within the same reproductive season. This study documents high variability in the migratory patterns of repeat spawners and high probability for them to stray, and suggests that straying is an important life-history strategy possibly affecting individual fitness.

Keywords: iteroparous, kelts, natal homing, repeat spawners, salmonids, straying.

INTRODUCTION

Natal philopatry, also referred to as natal homing, has been documented among a wide variety of taxa, including mammals, reptiles, birds, and fishes (Waser and Jones, 1983; Robichaud and Rose, 2001; Brothers and Lohmann, 2015). It is an evolutionary mechanism through which individual fitness is increased by having higher probability of finding suitable partners and habitat during the breeding season (Lohmann *et al.*, 2013; Mobley *et al.*, 2019), and through the development of local adaptations (Blair *et al.*, 1993; Keefer and Caudill, 2014).

Among salmonids, high natal homing tendency has been documented in different species and populations (Quinn, 1993; Klemetsen *et al.*, 2003; Keefer and Caudill, 2014), making them one of the classic examples for natal homing behaviour in the animal kingdom. Salmonids are noteworthy in that they have a high proportion of individuals within a population that home (Berg and Berg, 1987) and the ability to return to the specific stretch of river where they hatched with high accuracy (Quinn *et al.*, 1991; Vähä *et al.*, 2007).

However, there is often a proportion of the population that does not return to breed in its natal river, and instead strays to non-natal rivers (Quinn, 1993; Hendry *et al.*, 2004; Keefer and Caudill, 2014). Straying is an evolutionary mechanism, complementary to natal homing, through which individuals (re-)colonize new habitats. Further, straying may facilitate gene flow between populations (Schtickzelle and Quinn, 2007; Bekkevold *et al.*, 2020), which can, for example, decrease a population's susceptibility to environmental change. In general, qualitative assessments on straying rates among salmonid species have documented high variability in the proportion of

individuals that stray depending on the species and population (Berg and Berg, 1987; Jonsson *et al.*, 2003; Westley *et al.*, 2013). However, as there remains gaps in our knowledge of the mechanisms for natal homing and for that matter straying, it is difficult to determine the primary causes (e.g. failure to recognize natal river, evolutionary strategy, etc.) for the differences in the documented straying rates between species and populations. Past research has linked probability for individuals to stray to several factors, including for example origin (stocked vs. wild), environmental stability, and proximity of the natal and non-natal rivers (Jonsson *et al.*, 2003; Østergaard *et al.*, 2003; Keefer and Caudill, 2014). Nonetheless, there is a paucity of studies attempting to clarify why some individuals stray and others do not, and how the patterns of straying may change over multiple years of return among iteroparous salmonids.

Straying can have significant implications on population characteristics, including genetic structure and population dynamics (Bekkevold *et al.*, 2004; Massa-Gallucci *et al.*, 2010; King *et al.*, 2016). Several authors have documented that due to stable migration of individuals that successfully spawn in non-natal rivers, genetic differences, which are expected to be present between neighbouring rivers, have diminished (King *et al.*, 2016; Bekkevold *et al.*, 2020). This has further provoked speculations that multiple populations originating from different rivers may form a meta-population structure, caused by stable gene flow between the rivers (Østergaard *et al.*, 2003; Schtickzelle and Quinn, 2007). The effects of straying on population dynamics, however, have received less attention. For example, how does straying affect the demographics of spawners and subsequent recruitment? Does straying increase

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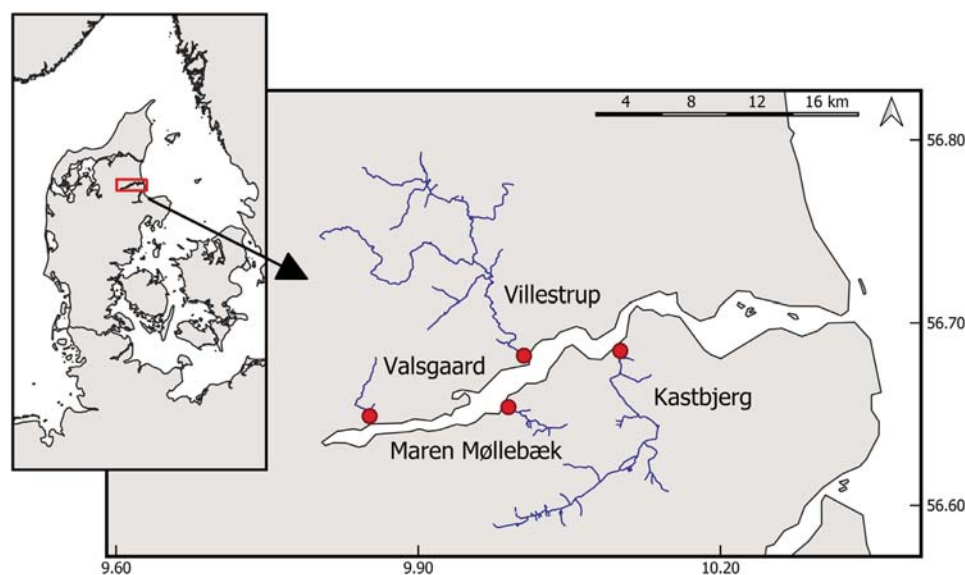


Figure 1. Juvenile out-migrating anadromous brown trout were tagged with PIT tags in the river Villestrup, and their subsequent return to freshwater was followed to their natal river Villestrup and non-natal rivers Kastbjerg, Valsgaard, and Maren Møllebæk. Migrations between the rivers and the sea were registered by PIT antennas (red dots) mounted close to the outlet of the rivers (Källo *et al.*, 2022).

Table 1. Total number of returning sea trout detected in any of the rivers in a given year, and total number of individuals returning to a non-natal river in a given year.

Year	Total number of sea trout returning*	Total number of sea trout straying**
1	1835 (8.5%)	663 (36.1%)
2	206 (11.2%)	55 (26.7%)
3	47 (22.8%)	8 (17.0%)
4	5 (10.6%)	0 (0%)
Total***	1835	677

* Percentage is calculated based on the total number of individuals that survived, and returned to freshwater from the year prior. For the first year, the percentage represents the proportion of individuals that were detected returning to any of the rivers after being tagged.

** Percentage is calculated based on the total number of sea trout that returned in a given year.

*** Total number of sea trout detected returning, and total number of strayers.

or decrease individual fitness? It is clear that straying can affect the population dynamics of both the donor and recipient populations, but the extent to which it does depends on the demographics of the strayers, and their success in contributing to the overall recruitment of the recipient river (Stacey *et al.*, 1997; Hendry *et al.*, 2004).

The implications of straying may be even more pronounced among iteroparous salmonids, where some individuals may return and attempt to spawn in non-natal rivers over multiple spawning seasons (Jonsson *et al.*, 2018). Repeat spawners have been documented to have disproportionately higher impact on the recruitment of the population, through higher fecundity and survival of offspring (Jonsson and Jonsson, 1999; Seamons and Quinn, 2010), compared to individuals returning to spawn for the first time (also known as maiden spawners). Life-history strategies of repeat spawners are understudied among iteroparous salmonids, thus our knowledge of the intrinsic and extrinsic factors that affect it is limited (Birnie-Gauvin *et al.*, 2019). This is further amplified for strayers; to our knowledge no studies of iteroparous salmonids have

followed migratory patterns of strayers over multiple years as the fish return to spawn.

The aim of this study was therefore to (1) document the proportion of strayers that return to freshwater for multiple times across years, (2) to determine whether variability exists in straying patterns over those years, and (3) to identify factors that may affect these patterns. To achieve this, anadromous brown trout (*Salmo trutta*), also referred to as sea trout, were tagged with Passive Integrated Transponder (PIT) tags as they out-migrated from one donor population as juveniles, and followed during their subsequent return to their natal and three additional non-natal (recipient) rivers for up to four years. In addition, all individuals were grouped according to their developmental status during out-migration, as parr, pre-smolt or smolt, in order to investigate how early life-history decisions may affect individual life-history during subsequent life stages. The current study is a follow-up study to Källo *et al.*, (2022), which focused on straying upon the first year of return from the sea.

METHODS

Study area and tagging protocol

Here a summary of the most important aspects of the methods used in this study is presented. A more detailed overview of the methods, as well as the results from the first return from the sea, are presented in Källo *et al.*, (2022).

This study took place in Mariager fjord, which is situated on the east coast of Jutland in Denmark (Figure 1). There are four main rivers that flow into the fjord and hold sea trout populations, which were monitored over the course of the study: Villestrup, Kastbjerg, Valsgaard, and Maren Møllebæk (Figure 1).

In 2015 and 2016, out-migrating juvenile brown trout were caught in the river Villestrup using a wolf-type trap (Wolf, 1951) and individuals larger than 11 cm were tagged with 23 mm PIT tags (Texas Instruments, RI-TRP-RRHP, half duplex, 134 kHz, length 23.1 mm, diameter 3.85 mm, and weight

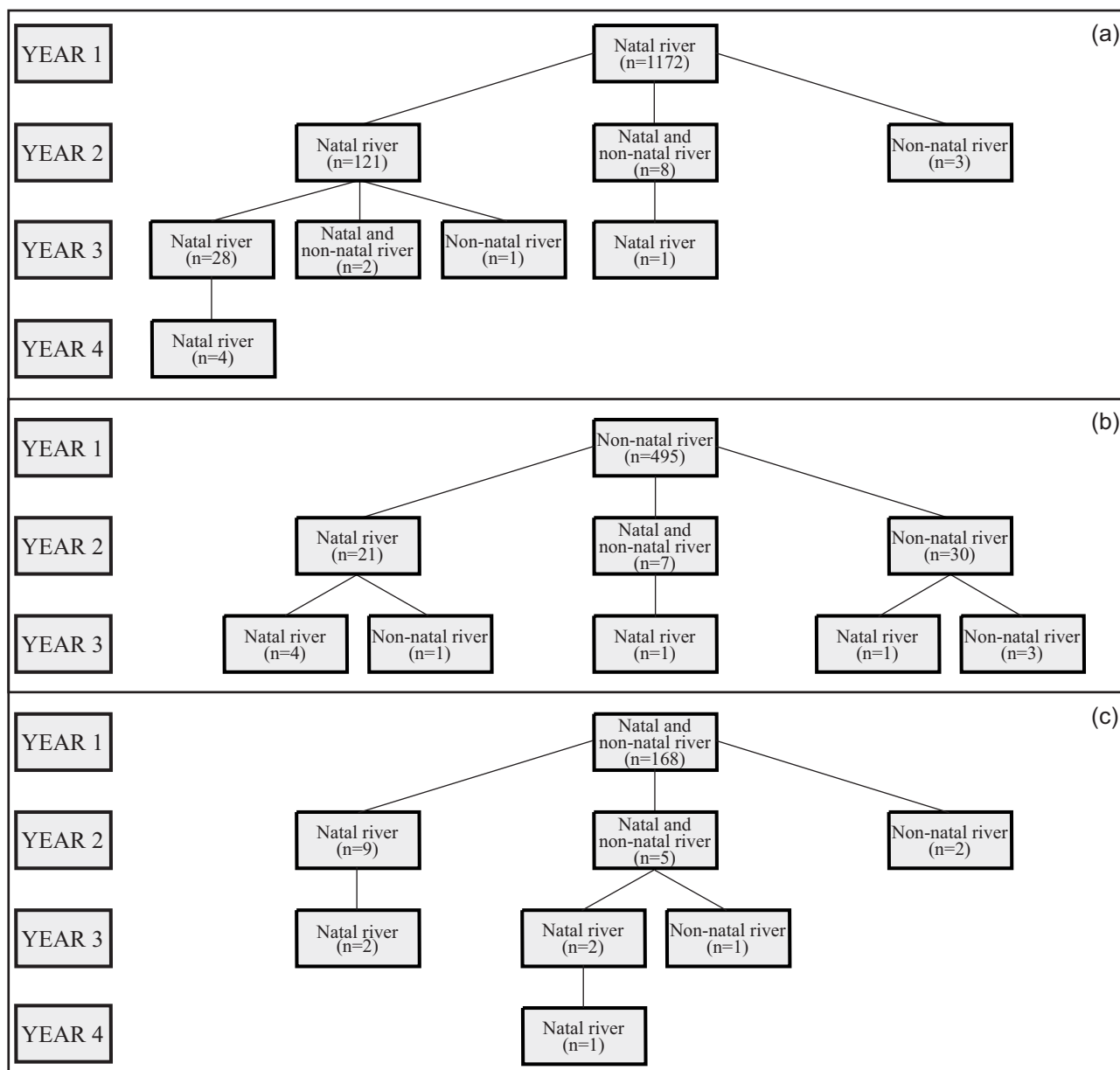


Figure 2. Migratory patterns of returning sea trout over multiple years, depending on whether they were detected in their (a) natal river only, (b) non-natal river(s) only, or (c) natal and non-natal rivers during the first year.

0.6 g in air). Prior to tagging, all individuals were anaesthetized with benzocaine (25 mg/l, Sigma Chemical Co., St Louis, USA) and subsequently weighed, measured in total length (TL) and grouped according to their developmental status as parr, pre-smolt and smolt (Tanguy *et al.*, 1994). The subsequent movements of individuals between freshwater and the sea were registered by double-antenna PIT systems (in order to determine the direction of movement), which were mounted at the outlet of each of the rivers. The antennas were working continuously from March 2015 until December 2021. The last download of PIT data was conducted in January 2022. The handling and tagging protocols used in this study have previously been shown to have minimum effects on individual survival and growth in salmonids (Acolas *et al.*, 2007; Larsen *et al.*, 2013).

Data handling and statistical analysis

Within the context of this study, individuals were considered strayers if they were detected in non-natal rivers Kastbjerg, Valsgaard, or Maren Møllebæk. As sea trout in this system return to freshwater at various times of the year, starting in early summer (Birnie-Gauvin *et al.*, 2021b; Källo *et al.*, 2022), all detections between 1st May until 30th April the following year are considered to be related to the same year of return. Herein, a “year” or “a year of return” will refer to the order of years each individual has been detected returning to freshwater, independent from year of tagging, and the length of time that had passed from the tagging event.

In order to determine whether migratory patterns were linked to possible spawning events, a spawning season was defined to last from 15th November until 31st December, as

Table 2. Number of returning sea trout that were only detected in their natal river, non-natal river(s) or both in their natal and non-natal river(s) across years. Percentages are calculated based on the total number of sea trout that returned for multiple years.

Group	Number of individuals
Natal river	118 (57.3%)
Non-natal river(s)	29 (14.1%)*
Natal and non-natal river(s)	59 (28.6%)**

* Three strayers were detected in multiple non-natal rivers, while the rest only returned to the same non-natal river.

** Seven strayers were detected in multiple non-natal rivers, while the rest only returned to the same non-natal river.

the majority of the spawning takes place within this period (Rasmussen and Pedersen, 2018). Therefore, individuals that had entered the river prior to 15th November without being detected leaving before that date, and individuals that entered the river between 15th November and 31st December were assumed to be in the river during the spawning season, with the aim to spawn. As a precautionary measure, all detections within the first 30 d post-tagging were removed, as it was not possible to determine if these individuals had left the river during that time (fish were released downstream of the antenna, which was roughly 400 metres from the outlet of the river). Further, individuals that did not follow the expected river-sea-river migratory pattern, meaning their migration between the river and the sea had been partially missed by the antennas, were removed from the dataset. This resulted in 26 individuals that were detected over multiple years being removed from the dataset. The estimates of return may be affected by possible tag expulsion, although studies on retention rates show high retention in salmonids, even over long time periods [533 d; (Acolas *et al.*, 2007; Foldvik and Kvingedal, 2018)].

To investigate the likelihood of individuals returning to freshwater over multiple seasons, and how this was affected by fish length (L_T), time spent at sea between tagging and initial return to freshwater (TaS; less than one year vs. more than one year), and whether a fish was a strayer or not (S), a Bernoulli distributed Generalized Linear Model (GLM) with logit link function was used. The original model included all two-way interactions of the aforementioned variables. Parsimonious model selection was conducted using AIC values (Zuur *et al.*, 2009). The final model fitted to the data was as follows:

$$\text{Spawning_season_}_{ij} \sim \text{Bern}(\pi_{ij})$$

$$\text{Logit}(\pi_{ij}) \sim \text{TaS}_{ij} + S_{ij} + L_{Tij} + \text{TaS}_{ij} \times S_{ij}$$

Further, a chi-square test was used to investigate differences in the number of individuals that returned over the years and survival rates between developmental groups, depending on if the individuals were detected only during one or multiple years. Fisher's exact test was used to investigate differences in the number of fish that had strayed or not in a given year. Data analysis and visualization were conducted in R (v 4.0.5, R Core Team, 2021).

RESULTS

A total of 21538 juvenile out-migrating sea trout were tagged in 2015 and 2016. Of those, 8.5% ($n = 1835$) were detected returning to rivers Villestrup, Kastbjerg, Maren Møllebæk, and Valsgaard in subsequent years, of which 37% ($n = 677$) were detected in a non-natal river. Of all the 1835 returning sea trout, 206 (11%) were detected over at least 2 years, including some for up to 4 years, with the percentage of individuals returning each year increasing over the first three years (Table 1).

Of these 206 individuals returning over multiple years, 43% ($n = 88$) were detected in non-natal rivers Kastbjerg, Valsgaard, or Maren Møllebæk at least once. These individuals will be referred to as strayers within the context of this study. The majority of the strayers (67%) that had returned to freshwater over multiple years displayed high variability in their migratory patterns across years (Figure 2). More specifically, the majority of strayers were detected in both their natal and non-natal rivers (Table 2), however within each year, the majority of them were only detected in non-natal rivers (Table 3). A similar trend is also present when only considering individuals that were detected returning to freshwater during the spawning season (Table 3). Further, the proportion of individuals that strayed in a given year decreased over the years, suggesting that the more years a sea trout returned to freshwater, the more likely it was to return to its natal river (Fisher's exact test; $p = 0.0005$, Table 1).

There was also a smaller number of strayers ($n = 29$) that were only detected in non-natal rivers across years, with the majority ($n = 26$) returning to the same non-natal river each year (Table 2). If considering these individuals and those that only return to natal river, then 70% of returning sea trout from this population returned ("homed") to the same river across all years, though not necessarily to their natal river.

Table 3. Number of individuals returning to their natal river only, non-natal river(s) only, or both in a given year of return. Numbers are further divided by total returns and returns during the spawning season. Percentages represent the proportion of fish in each group based on the total number of individuals detected in a given year, or within the spawning season.

Year of return	Fish returning to their natal river only in a given year		Fish returning to a non-natal river only in a given year		Fish returning to both their natal and non-natal river(s) in a given year			
	In total	During spawning season	In total	During spawning season	In total	During spawning season		
						Natal river	Non-natal river(s)	both
1	1172 (63.9%)	1049 (67.3%)	495 (26.9%)	346 (22.2%)	168 (9.2%)	109 (7.0%)	9 (0.6%)	46 (2.9%)
2	151 (73.3%)	146 (73.4%)	35 (17.0%)	33 (16.6%)	20 (9.7%)	14 (7.0%)	1 (0.5%)	5 (2.5%)
3	39 (82.9%)	35 (81.4%)	6 (12.8%)	6 (13.9%)	2 (4.3%)	2 (4.7%)	0	0
4	5 (100%)	4 (100%)	0	0	0	0	0	0

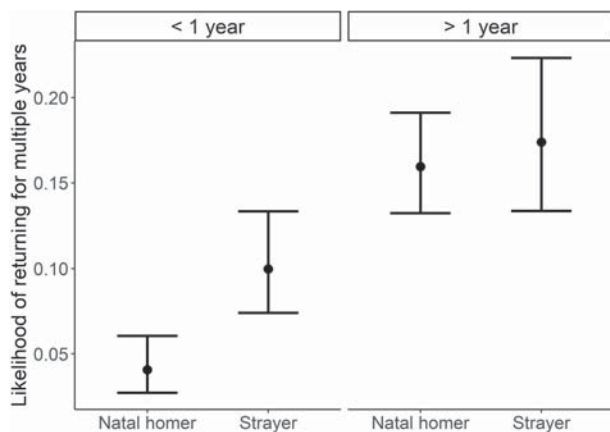


Figure 3. Modelled output of the likelihood of returning for multiple years against the duration of the first marine phase (<1 year vs. >1 year), among sea trout that strayed or homed. Sea trout that spent more than one year away at sea during their first marine phase were more likely to return for multiple years. The point represents the mean and the whiskers indicate the 95% confidence intervals. The plot is conditional on length fixed at mean LT = 145.5.

Table 4. Output of the most parsimonious GLM model where the likelihood of returning over multiple years was investigated.

Variable	df	Deviance	p-value
Time at sea	1	44.7	<0.001
Strayer	1	6.5	0.01
Length	1	0.5	0.5
Time at sea × Strayer	1	6.8	0.009

The bold entries represent variables where $p < 0.05$.

Factors affecting the individual likelihood to return over multiple years

According to the model, sea trout that spent longer than one year away at sea during their first marine phase were more likely to return multiple times compared to individuals that spent less than a year at sea (GLM; Time at sea; $F = 44.7$; $df = 1$; $p < 0.001$; Figure 3, Table 4). Furthermore, strayers had a higher probability of returning for multiple seasons compared to natal homers (GLM; Strayer; $F = 6.5$; $df = 1$; $p = 0.01$; Figure 3, Table 4), however this was only evident among individuals that had spent less than a year at sea (GLM; Time at sea × Strayer; $F = 6.8$; $df = 1$; $p = 0.009$).

Survival of sea trout from tagging until their first return to freshwater did not differ between smolts, pre-smolts, and parr (chi-squared; $\chi^2 = 2.7$, $df = 2$, $p = 0.3$; Table 5). However, the likelihood of the individuals to return for multiple years differed between groups (chi-squared; $\chi^2 = 12.6$, $df = 4$, $p = 0.01$). Smolts had a higher probability to return for at least two years, compared to parr (chi-squared; $\chi^2 = 4.1$, $df = 1$, $p = 0.04$) and pre-smolts (chi-squared; $\chi^2 = 8.2$, $df = 1$, $p = 0.004$). No differences were found between pre-smolts and parr (chi-squared; $\chi^2 = 0.4$, $df = 1$, $p = 0.5$).

DISCUSSION

Sea trout are known to display a continuum of life-history strategies at different developmental stages (Klemetsen *et al.*, 2003), although straying as a potential strategy has received

Table 5. Number of individuals in different groups classified according to their developmental status as parr, pre-smolt or smolt.

	Parr	Pre-smolt	Smolt
No. of individuals tagged	1668	10 256	9614
No. of individuals returning at least once*	154 (9.2%)	856 (8.3%)	825 (8.6%)
No. of individuals returning more than once**	10 (6.5%)	81 (9.5%)	115 (14.0%)

Percentages represent the proportion of individuals that survived from tagging until their first return* or from their first return until their second return**.

relatively less attention. In this study, we have documented high variability in patterns of straying during migration to freshwater over multiple years. To our knowledge, no other study has previously explored straying behaviour among sea trout individually followed over multiple years, making these results novel, and significantly contributing to our understanding of the behaviour of both strayers and repeat spawners.

Inter-annual variability in rates of natal homing and straying

In total, 11% of the sea trout that returned to freshwater were detected returning over multiple years, which is similar to those reported in previous studies (Jonsson and L'Abée-Lund, 1993; Kristensen *et al.*, 2019). Moreover, the proportion of individuals that successfully returned over multiple years increased over time, at least over the first three years. This is perhaps not surprising given that survival during the first marine phase (i.e. smolt to maiden spawner) is generally lower than that of repeat spawners (Jonsson and Jonsson, 2009). Further, even though spawning events were not directly observed, the majority of individuals had entered freshwater before or during the spawning season, and are therefore considered to have been there to spawn and will be referred to as repeat spawners. Alternatively, sea trout may ascend to freshwater during the winter, to escape adverse physiological conditions caused by high salinity and low temperatures (Thomsen *et al.*, 2007), however, this is considered to apply to a minority of returners in this system. Numerous electrofishing surveys have been conducted over multiple spawning seasons in this system, during which very few immature adults have been caught (unpublished data).

Among repeat spawners, originally tagged as juveniles in the river Villestrup, two contrasting migratory strategies were documented: a part of the population displayed a stable strategy and returned to the same river every year, while the other displayed a variable strategy, where they returned to different rivers over the years. Overall, the majority of the repeat spawners (~70%) only migrated to one of the monitored rivers. While most of them had only been to their natal river (natal homers), there was also a small proportion of strayers that had only migrated to the same non-natal river. Therefore, from a population's perspective, most repeat spawners in this population appear to display a stable strategy, and return to the same river over their lifetime, though not necessarily to the river they were born in.

This stable strategy; however, does not apply to all fish: strayers, which make up 43% of repeat spawners, generally returned to more than a single river, with 67% of them being detected in at least two rivers. Variability in migratory patterns was observed across years, with strayers visiting both natal and non-natal rivers, and individual strategy often changing from one year to next. However, within the year of return, individuals that strayed in a given year tended to only migrate to non-natal rivers, perhaps implying that once a “decision” to enter a specific river is made, individuals are unlikely to alter their strategy.

The results of this study further indicate that the more years strayers return to freshwater, the more likely they are to return to their natal river in later years rather than a non-natal river, which suggests that the likelihood to enter a specific river is not completely random. This may further indicate that there are specific intrinsic or extrinsic factors, such as condition, energetic status, or river characteristics, which are known to affect salmonid migrations (Klemetsen *et al.*, 2003; Thorstad *et al.*, 2008; Birnie-Gauvin *et al.*, 2021a), that can affect an individual's choice to return to a natal or a non-natal river, such that fitness is maximized.

As a result, this study provides evidence for an alternative hypothesis for the cause of straying. To date, most studies exploring straying rates and patterns among sea trout have originated from genetic studies (Bekkevold *et al.*, 2004; Massagallucci *et al.*, 2010; King *et al.*, 2016), where individual variability in behaviour cannot be observed nor considered. Further, the current understanding of natal homing implies that migration cues of the natal river are imprinted on fish as juveniles (Lema and Nevitt, 2004; Armstrong *et al.*, 2021), and it has been hypothesized that no additional learning processes happen subsequent to this stage (Hansen and Jonsson, 1994). This has resulted in a prevalent assumption that straying is caused by a failure to return to or recognize natal rivers (Schtickzelle and Quinn, 2007; Keefer *et al.*, 2014). In contrast, the findings from the current study display that natal homing (and straying) is a plastic life-history traits that is characterized by non-random change over the years, which may indicate that there is a decision-making process, possibly based on fitness-related trade off. However, the underpinning mechanisms for straying, as well as intraspecific variability in homing and straying, remain largely unknown, and may differ among populations (Dittman and Quinn, 1996; Moore *et al.*, 2007; Keefer and Caudill, 2014), so further research is needed to clarify this.

Although the proportion of sea trout that return to non-natal rivers across years represents a minority among all sea trout that return to spawn in this population, their impact on the recruitment, genetic structure, and other population parameters of the population(s) they return to should not be underestimated. Given that repeat spawners have higher fecundity (Jonsson and Jonsson, 1999) and spawning success (Serbezov *et al.*, 2012), they are likely to have a disproportionately high impact on recruitment (Stubberud *et al.*, 2021), and subsequently to the genetic structure and population dynamics of the river they return to compared to maiden spawners (Serbezov *et al.*, 2010; Bordeleau *et al.*, 2020). It has also been shown that repeat spawners significantly contribute to the persistence of stability in population abundance, especially in stochastic environment (Serbezov *et al.*, 2012; Bordeleau *et al.*, 2020; Stubberud *et al.*, 2021). This therefore implies that already a small number of individuals that stray over multiple spawning

seasons could significantly affect the population dynamics of both the donor and recipient population.

It should be noted; however, that in this study, not all rivers that the tagged sea trout may have migrated to were monitored. There are additional smaller rivers in the fjord, which were not monitored over the course of this study, but where the tagged fish could have migrated. However, given that the largest and most populated sea trout rivers in the area were the four rivers monitored in the study, and that only a small fraction of strayers return to the smallest river (Källo *et al.*, 2022), we find it unlikely that a significant number of tagged sea trout would have returned to these small non-monitored rivers. Nonetheless, we cannot exclude that some sea trout may have returned to non-monitored rivers outside of the fjord, as there is documentation that salmonids can stray to rivers relatively far away from their natal river (Birnie-Gauvin *et al.*, 2019). Regardless, we suggest that this applies only to a small number of individuals from the population, as recent genetic investigation into genetic differences between populations within and outside of Mariager fjord found clear genetic differences between the two areas, suggesting little to no gene flow between them (unpublished data, Källo *et al.*).

Factors affecting individual likelihood to return for multiple years

In order to determine possible factors that influence individual likelihood to return for multiple years, several life history characteristics were investigated. We found that the duration of the first marine phase had a significant effect on return probability: individuals that spent more than a year at sea during their first marine phase, were more likely to return for a second time. This is may be due to increased growth resulting from a longer marine phase (Jonsson and Jonsson, 2009; Birnie-Gauvin *et al.*, 2021b), as larger individuals tend to have higher survival (Aarestrup *et al.*, 2015; Kristensen, *et al.*, 2019). It was further documented that strayers have a slightly higher likelihood of returning for multiple years compared to natal homers; however, these differences were only evident among individuals that spent less than a year at sea.

In addition, return probability was dependent on developmental status during out-migration. Smolts were more likely to survive and return over multiple years compared to pre-smolts and parr, which may imply that out-migrating after having completed smoltification may have long-term consequences on individuals' survival. However, these findings contradict the results reported by del Villar-Guerra *et al.* (2019) about sea trout in the river Villestrup a few years earlier, which showed that parr had higher initial survival as maiden returners compared to pre-smolts and smolts. The present study found no difference in survival between developmental groups as maiden returners, while parr had the lowest probability to return for multiple years. Environmental stochasticity, which can significantly affect sea trout survival across years (Jonsson and Jonsson, 2009), may explain these differences, although long-term monitoring of sea trout survival in relation to environmental conditions is necessary to make that conclusion for certain.

Conclusions

Our findings reveal high variability in migratory patterns between multiple rivers among repeat spawners followed over several years, with straying as a possible strategy to increase

lifetime fitness. Even though, we documented that most repeat spawners display natal homing, and spawn in their natal river, there was a large proportion that did not do that. Approximately 43% of repeat spawners returned to a non-natal river(s), with the majority of them displaying variability in migratory patterns over the years. More specifically, the majority of strayers that return to spawn over multiple years migrated to both natal and non-natal rivers, and these migratory patterns change from one year to the next. While the underlying mechanisms determining which river sea trout return to are unclear, our results suggest this to be non-random, and as a result indicates that there may be a decision-making process, which is possibly influenced by fitness trade-offs. We hypothesize that straying repeat spawners may have significant implications for the genetic structure and population dynamics of both the recipient and donor populations. Therefore, managing these rivers as one meta-population may be an important conservation strategy, as the persistence of sea trout population in one river may be dependent on the persistence of sea trout populations in the others.

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Author contributions

All authors contributed to the conception and design of the study. KK and HB contributed to analysing the data. All authors contributed significantly to the interpretations of the results. All authors have given their final approval for the manuscript to be published.

Data availability

Data can be provided by the authors upon reasonable request.

Conflict of interest

Authors have no conflict of interest to declare.

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MANUSCRIPT III: On the factors affecting migration and straying in brown trout (*Salmo trutta*)

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On the factors affecting migration and straying in brown trout (*Salmo trutta*)

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Abstract

Anadromous brown trout display a continuum of life-history strategies with high intraspecific variability across their distribution range. While many of these strategies have been widely studied, there are some that have received proportionally less attention. In this study, we investigated intraspecific differences in two overlooked life-history strategies – out-migration phenology (spring vs autumn migrants) and the prevalence of straying – in brown trout originating from four rivers flowing into the same fjord system. Juvenile brown trout (*Salmo trutta*) were tagged with Passive Integrated Transponder (PIT) tags in their natal river, and were subsequently followed during out-migration and subsequent return to freshwater. We found that patterns of out-migration (spring vs autumn migrant, day of season) and return to freshwater (return likelihood, strayer vs non-strayers) differed between rivers, as did the factors (length, condition) affecting these outcomes. We also found evidence that out-migration phenology was connected to the likelihood of returning in a river-specific way. Our findings suggest that the costs and benefits of autumn migration and straying differ depending on an individual's home river.

Keywords: phenotypic plasticity, autumn migration, life-history strategies, phenology, dispersal

Introduction

Phenotypic plasticity enables organisms to modify their developmental trajectories, activities and resource allocation, typically in response to changes in environmental conditions (Pigliucci et al., 2006). For example, cyprinids in stream and reservoir habitats have been documented to differ morphologically in part due to flow variation (Franssen et al., 2013). Evidence also suggests that temperatures experienced during the embryonic stage affected later-developing life-history traits in Atlantic salmon (*Salmo salar*), including behavioral decisions like the timing of return from the ocean (Jonsson and Jonsson, 2019). Life-history traits like the timing of migration and age at maturity can be important determinants of fitness (del Villar-Guerra et al., 2019; Healey and Heard, 1984), and as such plasticity in these traits is likely an important aspect that ensures population sustainability. Within this context, brown trout (*Salmo trutta*) are particularly interesting because they display a high degree of polymorphism in their life-history strategies and migratory behavior (Ferguson et al., 2019).

The anadromous brown trout, also referred to as sea trout, is a migratory species, where individuals are born in freshwater and later migrate to marine environments to exploit greater food availability, aimed at increasing individual fitness (Klemetsen et al., 2003; Thorstad et al., 2016). Nevertheless, migration also entails costs, including energetic demands (Birnie-Gauvin et al., 2021; Boel et al., 2014; Ferguson et al., 2019; Forseth et al., 1999), and increased risk of mortality (del Villar-Guerra et al., 2019; Elliott, 1993; Källo et al., 2022a). Sea trout have been documented to display a continuum of life-history strategies and physiological traits during different stages of their life (Birnie-Gauvin et al., 2021; Cucherousset et al., 2005; Klemetsen et al., 2003). This includes inter- and intra-population differences in the timing of out-migration (Jonsson and L'Abée-Lund, 1993), level of smoltification during out-migration (del Villar-Guerra et al., 2019), size and age at out-migration and return (Thorstad et al., 2016), as well as migratory routes and habitat choice during the marine phase (Bordeleau et al., 2018; del Villar-

Guerra et al., 2014; Kristensen et al., 2019). Despite much attention being devoted to the study of brown trout life-histories, some important aspects of behavior have received less, including juvenile out-migration outside of peak periods, and dispersal to non-natal rivers (Birnie-Gauvin et al., 2019).

Juvenile brown trout have been observed migrating out of their natal river at various times of the year, with peaks in the spring and autumn (Jonsson and Jonsson, 2009). Yet, the majority of studies investigating the phenology and factors that affect out-migration have focused on spring migrants, with autumn migrants receiving far less attention (Birnie-Gauvin et al., 2019). This may stem from the perception that autumn migration appears to be somewhat maladaptive. For example, in Atlantic salmon, Riley et al. (2008) documented that autumn migrants were not physiologically prepared for sea entry. Moreover, Jonsson and Jonsson (2009) documented that autumn migrants had lower return rates compared to spring migrants in sea trout. In contrast, a more recent investigation documented that this may not always be the case; autumn-migrating sea trout from River Gudsø, Denmark, had similar return rates to their spring-migrating counterpart (Birnie-Gauvin and Aarestrup, 2019). Together, these findings indicate that the benefits and/or costs of autumn migration may differ between species and populations. The study of autumn migration has increased over the last decade, as the strategy and its potential impacts on population dynamics are increasingly recognized (e.g., Aarestrup et al., 2018; Birnie-Gauvin et al., 2021; Kennedy et al., 2022; Taal et al., 2014; Winter et al., 2016). However, our understanding of the factors that drive individuals to migrate in spring or autumn, and how this decision is connected to subsequent life-history traits (e.g., survival and spawning) is limited.

Subsequent to out-migration and the marine phase, sea trout are expected to return to their natal river for spawning, as high natal homing capabilities have been documented among salmonids (Hendry et al., 2004; Keefer and Caudill, 2014; Quinn, 1993). Impressively, some may even

return to the specific stretches of river where they hatched (Quinn et al., 1991; Vähä et al., 2007). However, not all individuals return to their natal river; in some instances, a proportion of returning individuals stray, and return to non-natal rivers to spawn (Degerman et al., 2012; Källo et al., 2022a). Although straying in salmonids has been qualitatively documented through genetic analyses in several systems (Bekkevold et al., 2020; King et al., 2016), studies providing quantitative assessments of straying and that explore the factors that affect its propensity are scarce.

Recent evidence suggests that straying in brown trout can be highly prevalent (Källo et al. 2022a; Masson et al., 2017), with patterns of straying and the factors that affect them likely varying across systems. For example, straying has been documented to decrease with distance from the natal river (Berg and Berg, 1987; Jonsson et al., 2003), and increase towards larger rivers (Degerman et al., 2012; Unwin and Quinn, 1993). The causes for these patterns remain unclear, as it is often unknown why some individuals stray and others do not. However, findings that stocked fish stray to a higher degree (Jonsson et al., 2003) have led to the general assumption that straying may be maladaptive and likely caused by a failure in natal imprinting. On the other hand, straying has also been linked to several life-history characteristics, like length at out-migration (juveniles), developmental status (parr, pre-smolt, smolt) and the timing of out-migration (Källo et al., 2022a). The presence of such patterns suggests that rather than representing a maladaptation or a failure to properly imprint, straying may instead represent a life-history strategy serving to increase individual fitness in part of the population (Källo et al., 2022a). Moreover, patterns of straying have often been documented from one donor population to one or more recipient populations (Berg and Berg, 1987; Jonsson et al., 2003), generally using various sampling intensities. Thus, straying has generally been assessed in only one direction, limiting the complete assessment of the spatial patterns of straying between all possible rivers that strayers may migrate to, as well as the factors that affect these patterns.

Consequently, further documentation of the patterns of straying and how they connect to various life-history characteristics between multiple donor and recipient populations is required.

To address the current knowledge gaps related to autumn migration and straying, the aim of this study was to investigate intraspecific differences in out-migration phenology (autumn vs spring migration) and to quantify the prevalence of straying among trout originating from four nearby rivers that flow into the same fjord system (i.e., they share the same marine habitat). To do so, juvenile brown trout were tagged with Passive Integrated Transponder (PIT) tags over two years in all four rivers, and their out-migration and return to freshwater were followed to both natal and non-natal rivers.

Materials and Methods

Study site

This study took place in Jutland, Denmark, where juvenile brown trout were tagged prior to their out-migration in four different rivers: 1) Villestrup, 2) Kastbjerg, 3) Valsgaard and 4) Maren Møllebæk (Figure 1). While the age of the tagged individuals was not determined, based on earlier studies (Rasmussen and Pedersen, 2018) it is likely that most tagged individuals were 1+ or 2+ fish, that had not previously out-migrated from a given river. The rivers investigated in this study differed in length and surface area with River Villestrup being the largest, followed by rivers Kastbjerg, Valsgaard and Maren Møllebæk (see Table 1 for details). All the rivers flow into Mariager Fjord, which is connected to the Kattegat Sea in the east.

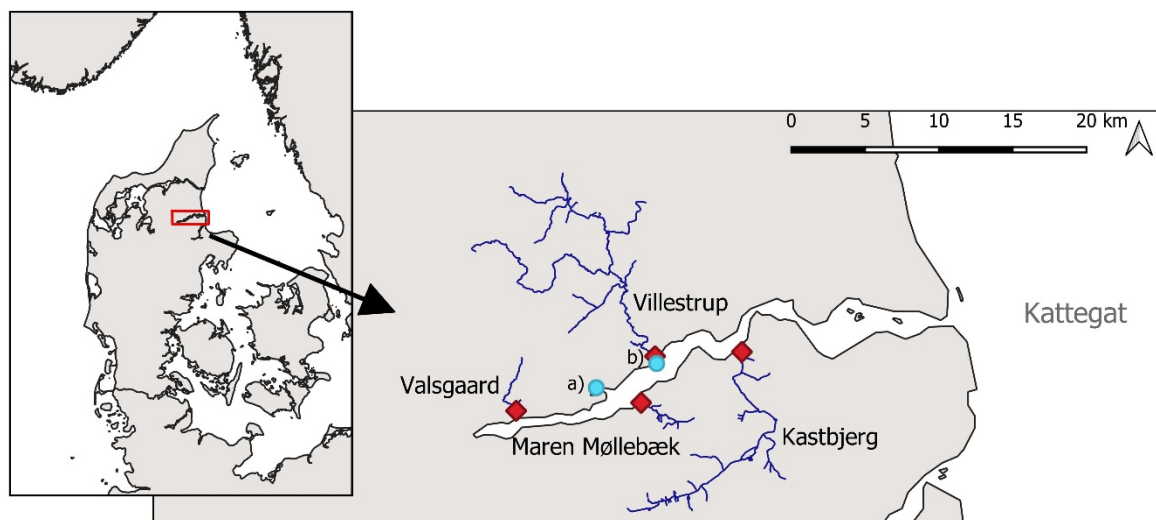


Figure 1. Juvenile brown trout were tagged in rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk prior to out-migration. Their migratory patterns in and out of their natal and non-natal rivers were followed with PIT-antennas (red diamonds), mounted at the outlet of each of the rivers. To determine predation rate by cormorants, two cormorant colonies (blue circles), named Kielstrup (a) and Villestrup (b) were scanned.

Fish capture and tagging

Juvenile brown trout were caught via electrofishing in each of the four rivers in early July of 2018 and 2019 (see Table 2 for details). Approximately 500 fish (>11.0 cm) were captured from each river in each year, except for Maren Møllebæk (2018 $n=318$; 2019 $n=179$) where too few fish could be found. Upon capture, fish were immediately transferred to a bin with oxygenated fresh stream water. Fish were then anesthetized with benzocaine (25 mg/l, Sigma Chemical Co., St Louis, USA) to be measured for length (± 0.1 cm) and mass (± 0.1 g), and subsequently tagged with a 23-mm Passive Integrated Transponder (PIT) tag (Texas Instruments, RI-TRP-RRHP, half duplex, 134 kHz, length 23.1 mm, diameter 3.85 mm, and weight 0.6 g in air). PIT tags were inserted lateroventrally, posterior to the pectoral fin, through a 2-mm incision. Fish were subsequently returned to a bin with oxygenated fresh stream water to recover. Once fully recovered, fish were released near their site of capture. The Danish Experimental Animal Committee (2017-15-0201-01164) has approved all protocols used in this study.

Table 1. The main characteristics of rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk.

	Villestrup	Kastbjerg	Valsgaard	Maren Møllebæk
Length (km)	21	21	7	5
Width (m)	4 – 12	3 – 8	0.5 – 3	0.5 – 2
Depth (m)	0.5 – 3	0.3 – 2	0.1 – 1	0.01 – 0.3
Mean annual discharge \pm SD (m^3s^{-1})	1.6 ± 0.3	0.8 ± 0.3	0.1 ± 0.02	NA
Mean annual temperature \pm SD ($^{\circ}\text{C}$)	8.9 ± 3.0 $^{\circ}\text{C}$	9.9 ± 4.4 $^{\circ}\text{C}$	9.1 ± 3.2 $^{\circ}\text{C}$	9.8 ± 2.6 $^{\circ}\text{C}$
PIT antenna efficiency ^a	59.6% /97.4%	78.9% /86.4%	85.2% / 100%	75.2% /100%

^a Efficiency of the upstream antenna / efficiency of the down-stream antenna

Table 2. Tagging information. Dates of tagging, number of fish, average length \pm SD (cm), mass \pm SD (g) and condition \pm SD for each river system.

Year	Villemstrup		Kastbjerg		Valsgaard		Maren Møllebæk	
	2018	2019	2018	2019	2018	2019	2018	2019
Tagging dates	4 – 7 July	16 – 17 July	16 – 18 July	7 – 8 July	9 – 10 July	2 – 3 July	11 – 12 July	1 July
N	502	500	493	500	497	500	318	179
Length (cm)	12.9 \pm 1.2	12.9 \pm 1.3	13.3 \pm 1.2	13.8 \pm 1.3	12.7 \pm 1.2	12.4 \pm 1.1	12.6 \pm 1.1	12.5 \pm 1.2
Mass (g)	23.1 \pm 6.9	23.2 \pm 7.6	24.2 \pm 6.8	27.4 \pm 8.3	21.6 \pm 7.1	20.5 \pm 6.3	19.9 \pm 5.8	20.3 \pm 6.7
Condition	1.04 \pm 0.06	1.05 \pm 0.07	1.00 \pm 0.06	1.00 \pm 0.07	1.03 \pm 0.07	1.05 \pm 0.08	0.98 \pm 0.06	1.00 \pm 0.05
Total	1002		993		997		497	

Migration and straying

Movements of individuals between the fjord and the river were followed by double PIT-antenna systems mounted approximately 200-400 meters upstream from the outlet of each of the rivers. Efficiency for both upstream and downstream antenna for each river was calculated according to Zydlewski et al. (2006) (Table 1). Fish were considered to have migrated out to sea if they were detected at both the upstream and downstream antenna, in that order. Some fish may have not been detected upon out-migration at all or by only one antenna, but were considered to have migrated to sea if (1) they were detected returning (see below) to their natal river at least 90 days after tagging or from their last detection; or (2) they were detected entering a non-natal river independent of the length of time that had passed since tagging. Date of out-migration was not available for fish not detected upon out-migration, but was otherwise considered as the date of the last detection during out-migration. All out-migrating individuals for which date of out-migration was available were assigned as spring or autumn out-migrants. While out-migration from the natal river took place continuously throughout the year, there were two peaks: one during autumn and one during spring. The autumn peak lasted from August until the end of December, and the spring peak lasted from January until the end of May (Figure 2). Based on the latter, juvenile brown trout that left their natal river between 1 July and 31 December or 1 January and 30 June were grouped as autumn and spring migrants, respectively. Unfortunately, due to high water discharge following a flood in river Villestrup and Kastbjerg in spring 2020, the antennas in those rivers had, for a limited time, low detection efficiency, resulting in the underestimation of individuals out-migrating from rivers Villestrup and Kastbjerg in spring 2020.

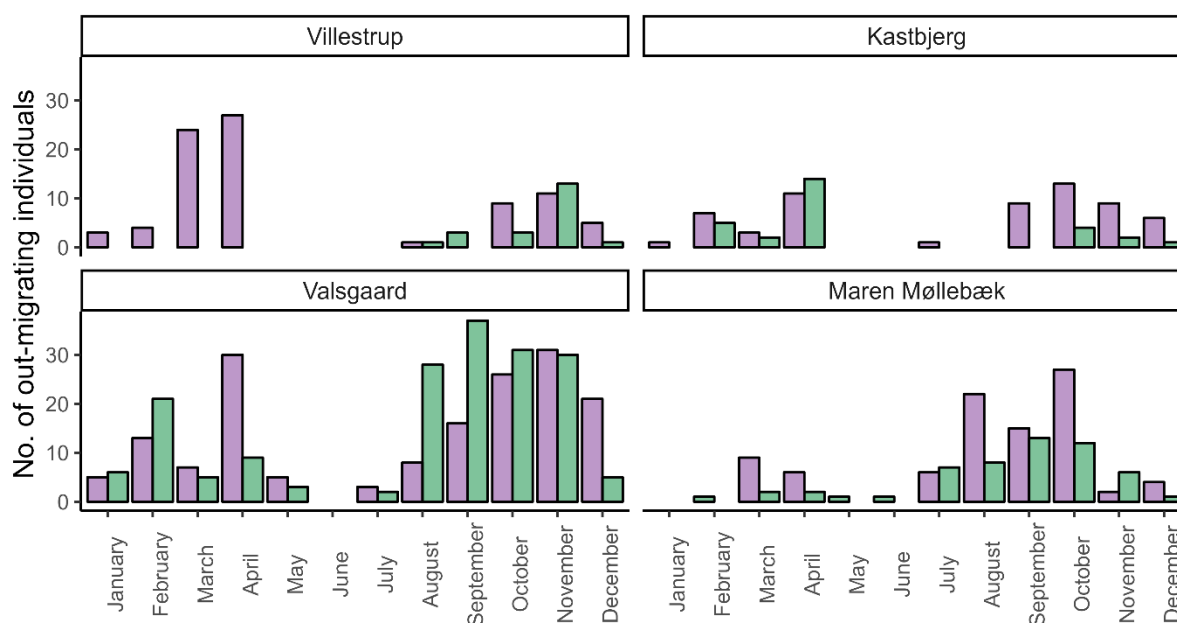


Figure 2. Number of individuals out-migrating from their natal river across months. Different colors represent individuals tagged in 2018 (purple) and 2019 (green).

Sea trout were considered to have returned to a river if they were detected at both the downstream and upstream antennas, in that order. Date of return was considered as the first detection at the downstream antenna. The length of the marine phase was calculated based on the number of days between the date of out-migration from the natal river and the date of return to a natal or non-natal river. Individuals detected returning to freshwater within 30 days from out-migration were excluded from the analysis ($n=13$) since we do not consider 30 days to be a true marine migration, but may instead represent movements just below the PIT antennas. Fish were considered to have strayed if they were detected in a river other than the one they were tagged in. Individuals were considered to be in freshwater to spawn if they were detected there during the spawning season, which was defined to last from 15 November until 31 December (Rasmussen and Pedersen, 2018). Sea trout may also ascend to freshwater as immature fish (Thomsen et al., 2007), although this is considered to apply to a minority of individuals in this system because very few immature fish have been caught, at least in river

Villestrup, during spawning population surveys. The PIT-antennas were operational in all the rivers from July 2018 until January 2022, when the last download of PIT-data was conducted. The PIT-antennas were operational over a time period when majority of tagged individuals are expected to out-migrate and subsequently return to freshwater.

Predation

To estimate predation rate among brown trout tagged in this study, two near-by cormorant colonies, named Villestrup and Kielstrup (described in Källo et al. 2020), were scanned for PIT-tags using a portable scanner (Skov et al., 2014) in spring 2022 (Figure 1). Predation rate was calculated by correcting the raw tag counts with detections efficiencies that have been previously reported for these colonies (Källo et al., 2020).

Data analyses

The two main principles applied in this study during the model building process were (1) that all initial models included all main effects of the named variables and all possible two-way interactions, and (2) that the most parsimonious model selection was used, meaning that the final model the data was fitted to was selected based on backwards model selection using AIC values (Zuur et al., 2009).

Multiple Generalized Linear Models (GLM), which are presented in Table 3, were used to investigate different factors affecting out-migration, return, and straying among the tagged individuals. Out-migration type refers to whether an individual was detected out-migrating or not, out-migration phenology refers to whether an individual was grouped as spring or autumn out-migrant, straying status refers to whether an individual strayed or not, and predation status refers to whether an individual was predated or not. Condition (Fulton's K) of individuals was

calculated based on the following formula: $\text{Condition (K)} = \frac{\text{mass (g)}}{(\text{length (cm)})^3} \times 100$.

Table 3. Details of the final Generalized Linear models used in this study subsequent to model selection process aimed to investigate differences in factors affecting out-migration, return and straying.

Model ID	Distribution	Dependent variable ~ Independent variables
General		
GLM 1a	Gamma	Length ~ River of tagging + Year of tagging + River of tagging \times Year
GLM 1b	Gamma	Condition ~ River of tagging + Year of tagging + River of tagging \times Year
Out-migration		
GLM 2a ^a	Bernoulli	Out-migration type ~ River of Tagging + Length
GLM 2b ^a	Bernoulli	Out-migration type ~ River of Tagging + Condition
GLM 3a	Bernoulli	Out-migration phenology ~ River of tagging + Length + River of tagging \times Length
GLM 3b	Bernoulli	Out-migration phenology ~ Condition + River of Tagging
GLM 4	Gamma	Day of season (autumn) ~ River of tagging + Year of tagging + Length + River of tagging \times Year of tagging
Return		
GLM 5	Gamma	Days at sea ~ River of tagging + Out-migration phenology + Straying status
GLM 6	Bernoulli	Return type ~ River of tagging + Out-migration phenology + Length + River of tagging \times Out-migration phenology
GLM 7	Bernoulli	Straying status ~ River of tagging + Out-migration phenology + Length
GLM 8	Bernoulli	Predation status ~ River of tagging+ Length+ Year of tagging
GLM 9 ^b	Bernoulli	Predation status ~ River of tagging+ Length+ Year of tagging+ Out-migration phenology

^a Only trout tagged in 2018 were included in the analysis

^b Only individuals that out-migrated from their natal river were included in the analysis

Results

River-specific differences at the time of tagging

In total, 3489 juvenile brown trout were tagged in rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk in July 2018 and 2019, prior to out-migrating from their natal river (Table 2). The length of juvenile brown trout differed among all rivers (GLM 1a; River of tagging; $F=144.8$; $df=3$; $p<0.001$, Figure 3), except between Valsgaard and Maren Møllebæk, and further varied between the years of tagging (GLM 1a; River of tagging \times Year of tagging; $F=18.4$; $df=3$; $p<0.001$, Figure 3). There were also river-specific differences in condition (GLM 1b; River of tagging; $F=156.5$; $df=3$; $p<0.001$, Figure 3), which further differed between years (GLM 1b; River of tagging \times Year; $F=6.7$; $df=3$; $p<0.001$, Figure 3).

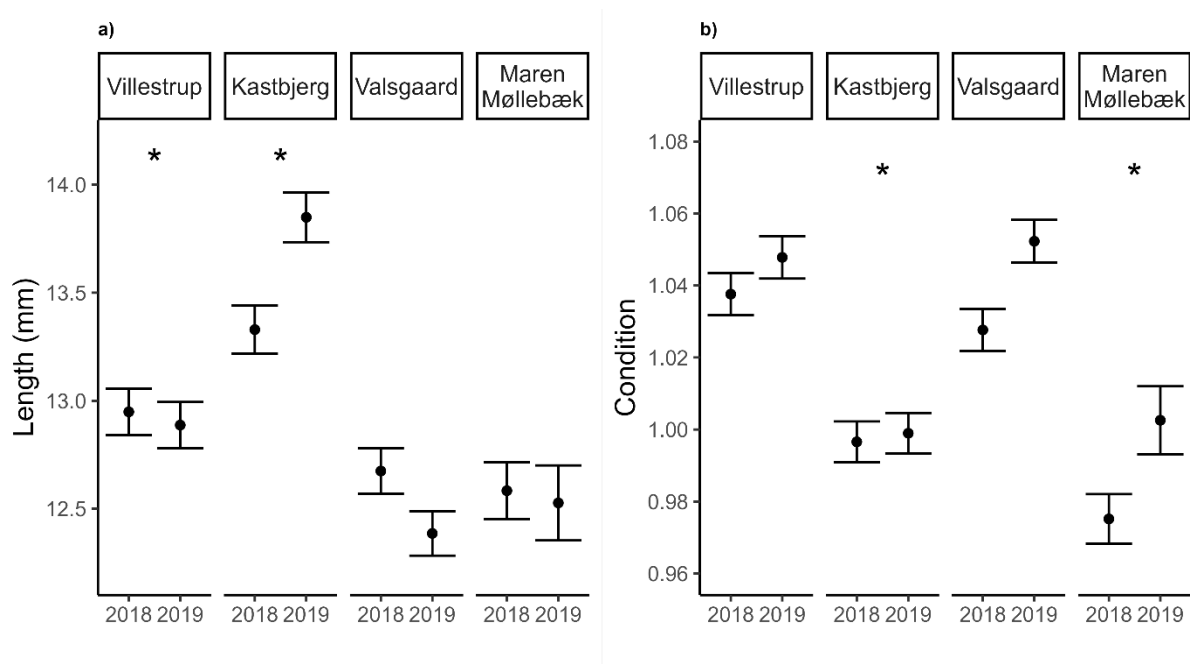


Figure 3. Modelled output of length (a) and condition (b) against year of tagging in different rivers. There were differences between rivers and years in length (a) and condition (b) during tagging. The asterisk represent the rivers that significantly differed from *all* rivers in length (a) or condition (b). The points represent mean estimates and the whiskers 95% confidence intervals.

Out-migration of juveniles

In total, 680 (19.5%) individuals were detected migrating out of their natal river. This is a minimum estimate, as flooding in river Villestrup and Kastbjerg in the spring of 2020 caused a reduction in the antenna detection efficiency, decreasing the number of juveniles detected migrating out to sea. Consequently, the likelihood for juveniles to migrate out of their natal river was only investigated for individuals tagged in 2018 (in all rivers). We found that in 2018, juvenile brown trout originating from the two smaller rivers, Valsgaard and Maren Møllebæk, had a higher likelihood to out-migrate compared to those from Villestrup and Kastbjerg (GLM 2a; River of tagging; $\text{Chi}=79.5$; $\text{df}=3$, $p<0.001$, Table 4). The length (GLM 2a; Length; $\text{Chi}=1.7$; $\text{df}=1$; $p=0.2$) and condition (GLM 2b, Condition; $\text{Chi}=1.9$; $\text{df}=1$; $p=0.2$) at tagging had no effect on individual likelihood to out-migrate.

In total, 453 (67%) and 227 (33%) juveniles out-migrated during autumn and spring, respectively (Table 4). Again, we note that the number of spring migrants tagged in 2019 is underestimated due to a low detection efficiency of the PIT antenna during the spring of 2020 caused by flooding. However, even when only considering individuals tagged in 2018, more individuals were detected out-migrating in the autumn ($n=245$, 61%) compared to spring ($n=155$, 39%).

Table 4. Number of brown trout PIT-tagged in 2018 and 2019 that out-migrated from Rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk. Out-migrated fish are further divided into autumn or spring migrants depending on the timing of out-migration. Presumed migrants represent the individuals that were not detected out-migrating from their natal river but were detected returning to any of the rivers within the fjord (i.e., known migrants with unknown out-migration timing).

	Villestrup		Kastbjerg		Valsgaard		Maren	
	2018	2019	2018	2019	2018	2019	2018	2019
Initial sample size	502	500	493	500	497	500	318	179
Out-migrated ^a	84	21	60	28	165	177	91	54
	(16.7 %)	(4.2 %)	(12.2 %)	(5.6 %)	(33.2 %)	(35.4 %)	(28.6 %)	(30.2 %)
Autumn migrants ^b	26	21	38	7	105	133	76	47
	(30.9 %)	(100 %)	(63.3 %)	(25 %)	(63.6 %)	(75.1 %)	(83.5 %)	(87.0%)
Spring migrants ^b	58	0	22	21	60	44	15	7
	(69.0 %)		(36.7%)	(75 %)	(36.4 %)	(24.9 %)	(16.5 %)	(13.0 %)
Presumed migrants	1	12	1	8	1	1	0	0
Returned to freshwater	10	24	13	17	27	25	18	11

^a Percentage is based on the total number of fish that were tagged in a given river and given year

^b Percentage is based on the total number of individuals detected out-migrating of their natal river among fish tagged in a given river and given year.

We found that the probability for individuals to migrate in autumn or spring depended on the river of tagging (GLM 3a; River of tagging; $\text{Chi} = 60.3$; $\text{df} = 3$; $p < 0.001$; Table 4), as well as individual length, though this effect differed between rivers (GLM 3a; River of tagging \times Length; $\text{Chi} = 9.3$; $\text{df} = 3$; $p = 0.03$; Figure 4). The effect of length was most pronounced in the two larger rivers, Villestrup and Kastbjerg, while it appeared negligible in Valsgaard and Maren Møllebæk (Figure 4). In all the rivers, condition affected whether fish migrated in autumn or spring (GLM 3b; Condition; $\text{Chi} = 8.7$; $\text{df} = 1$; $p = 0.003$), with spring migrants having a higher condition than autumn migrants (Figure 4).

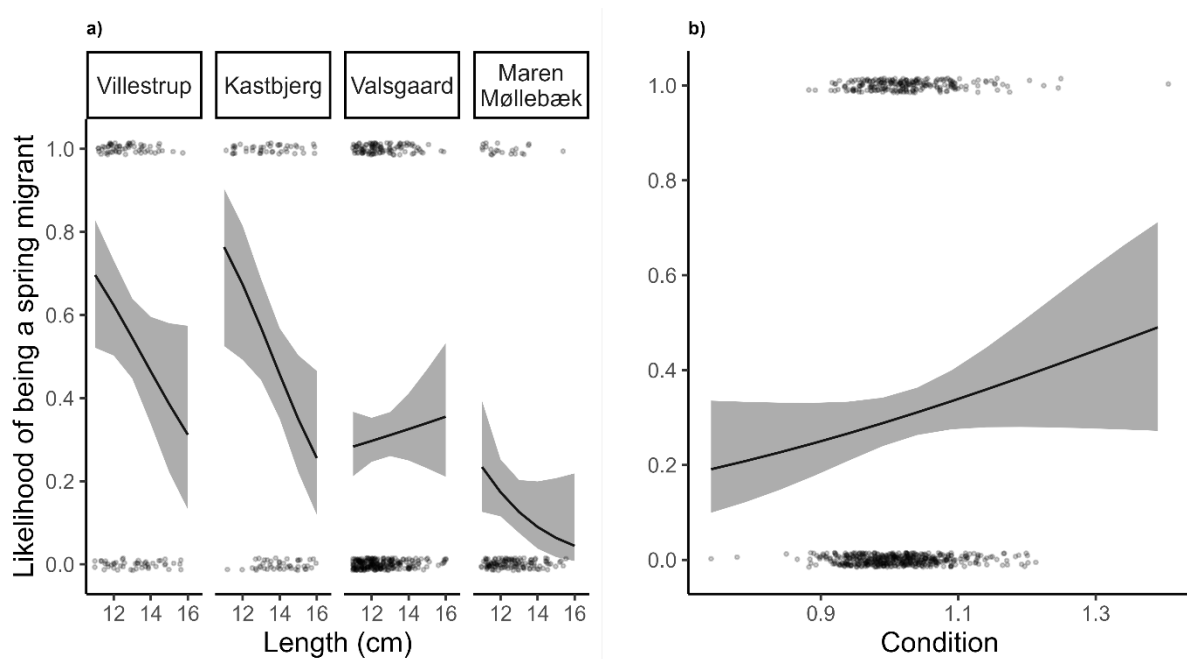


Figure 4. Modelled output of the likelihood of being a spring migrant (as opposed to autumn migrant) as a function of length at tagging (cm) (a) or condition (b). The likelihood of being a spring migrant depends on individual length and the river of origin (a) and condition (b), independently. The lines and the shaded areas represent the mean and the 95% confidence intervals of the model output. The points represent individual out-migrating brown trout, depending on their length (a) or condition (b), with “0” representing autumn migrants and “1” representing spring migrants. Plot b) is conditional on River of tagging = Valsgaard.

Further, within-season differences in the timing of out-migration between the rivers were only evident among autumn migrants (GLM 4; River of tagging; $F=22.6$; $df=3$; $p<0.001$), and further varied between years (GLM 4; River of tagging \times Year of tagging; $F=2.7$ $df=3$, $p=0.04$; Figure 5). The differences between years were most evident in river Valsgaard, where autumn-migrating juveniles in 2019 out-migrated 34 days earlier than in 2018. Overall, based on the median day of out-migration individuals from Maren Møllebæk migrated the earliest in the season, which was followed by Valsgaard, Kastbjerg and Villestrup. However, pair-wise comparisons only revealed that autumn migrants from Maren Møllebæk migrated significantly earlier compared to all the other rivers.

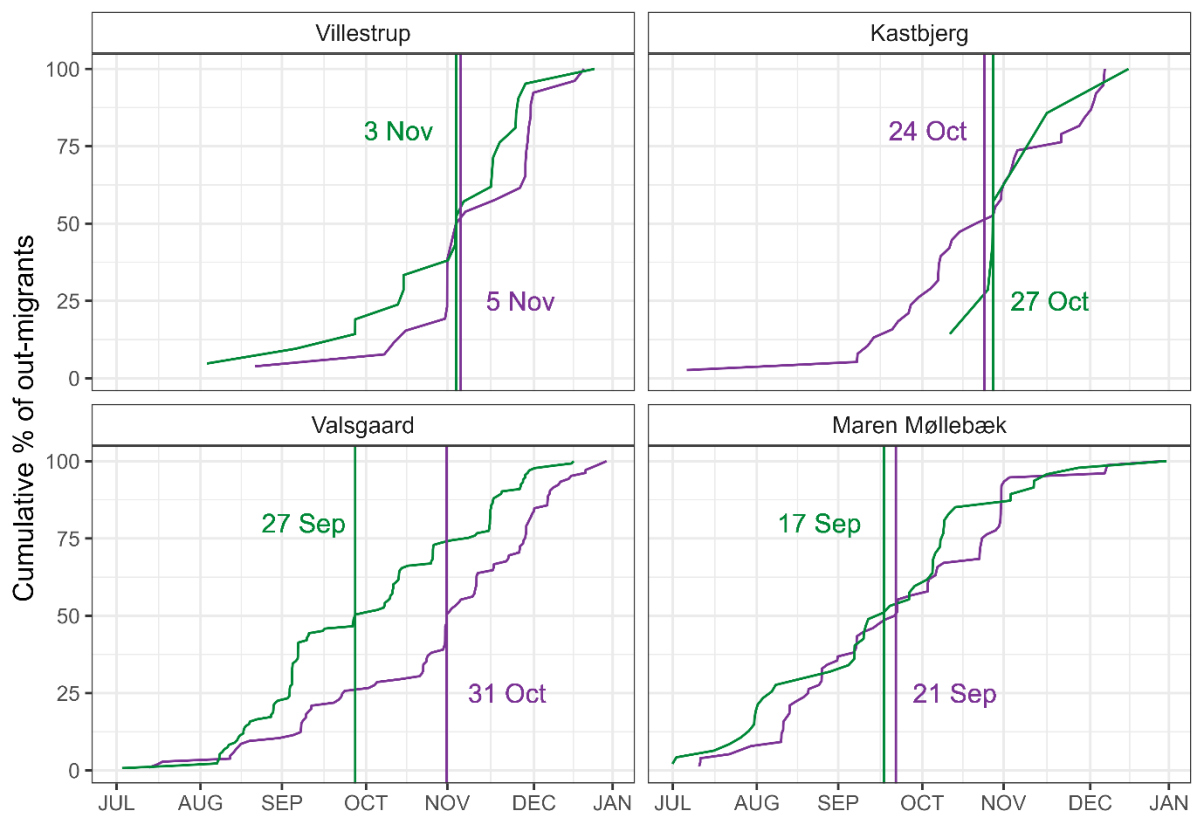


Figure 5. Cumulative percentage of out-migrating brown trout tagged in 2018 (Purple) and 2019 (green) in the autumn with vertical line and the date representing the median day of out-migration in a given river and year.

Return to freshwater

A total of 145 fish were detected returning to any of the four monitored rivers. Among those were 24 individuals that were not detected leaving their natal river, but that were either detected entering it at least 90 days post-tagging, or entering a non-natal river. When only including individuals detected both upon out-migration and return, the return rate to any of the four rivers was 18% (n=121; Table 5). Overall, there were two main peaks in the timing of return to freshwater: during summer (July-September) and autumn (October-January). There was high variability in the length of the marine phase, ranging from 31 days to several years (maximum 870 days), with the time spent at sea being longer for autumn migrants than spring migrants (GLM 5; Out-migration phenology; $F=6.3$; $df=1$; $p=0.01$; Table 5). Autumn migrants spent a median of 399 days at sea, while spring migrants spent a median of 216 days at sea.

Table 5. Number of sea trout that returned in total and specifically to non-natal river(s) (in total and grouped by the year of tagging), depending on which river they were tagged in, along with the median (min – max) days spent at sea for those respective groups. Return rate is calculated based on the individuals that were detected both out-migrating and returning.

River	No. of returners in total	Overall return rate (2018/2019)	No. of strayers		Median days spent at sea (min-max)	
			In total ^a	2018/2019 ^b	Natal homers	Strayers
Villestrup	34	20 % (11% / 57%)	17 (50 %)	4 (40%) / 13 (54%)	356 (172-651)	113 (51-667)
Kastbjerg	30	24 % (20% / 32%)	4 (13 %)	2 (15%) / 2 (12%)	286 (104-870)	399 (247-646)
Valsgaard	52	15 % (16% / 14%)	19 (37 %)	10 (37%)/ 9 (36%)	383 (166-775)	303 (50-809)
Maren	29	20% (20% / 20%)	13 (45 %)	7 (39%) / 6 (55%)	437 (148-864)	117 (31-642)
Møllebæk						

Individual likelihood to return to freshwater was dependent on the river of origin and the timing of out-migration (GLM 6; River of origin \times Timing of out-migration; Chi=37.1; df=3; $p<0.001$; Figure 6). Spring migrants from Kastbjerg and Maren Møllebæk were more likely to return compared to autumn migrants from the same rivers, while the opposite pattern was documented in river Villestrup. There was no effect of timing of out-migration on likelihood of return in river Valsgaard.

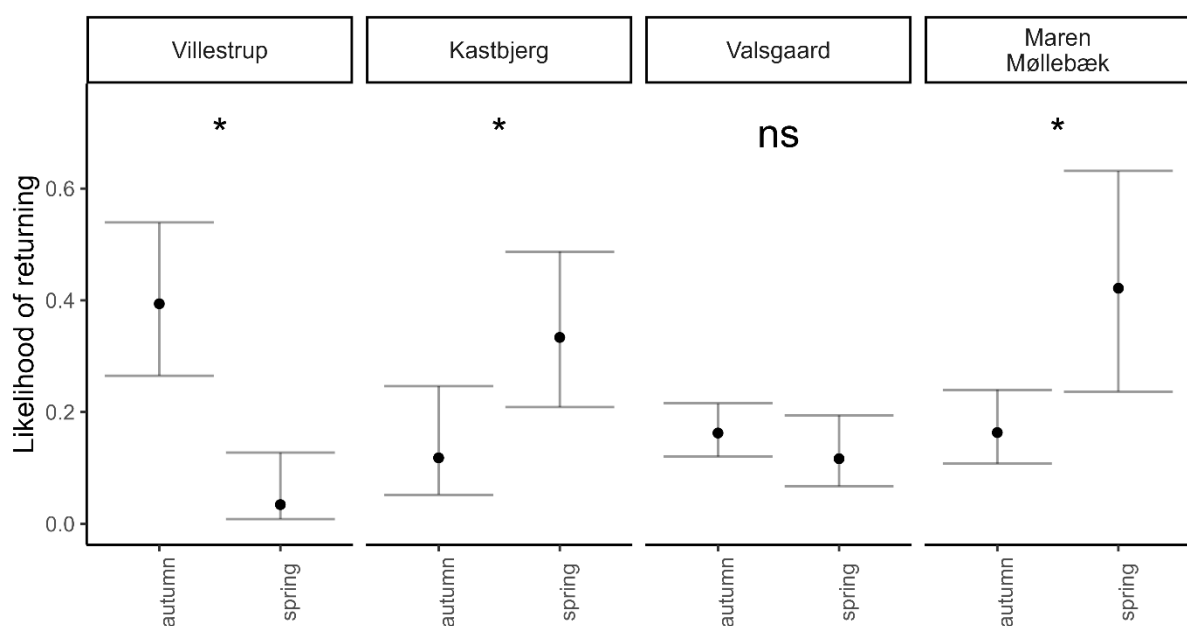


Figure 6. Modelled output of the likelihood of returning as a function of out-migration phenology (autumn vs spring out-migrants) and the river of tagging. The points and whiskers represent the mean and the 95% confidence intervals of the model output, respectively. The asterisks refer to rivers, where there were significant differences between autumn and spring migrants in their likelihood of returning, while “ns” refers to non-significant differences.

Patterns of straying

Of all returning sea trout, 53 (37%) were detected in non-natal rivers and are herein referred to as strayers. Most strayers (n=38) were only detected in one non-natal river, though one sea trout was detected in two non-natal rivers. A smaller group of individuals (n=15) returned to

both natal and non-natal rivers. The majority of the strayers (66%) were in a non-natal river during the spawning season. River of origin did not have an effect on the likelihood of individuals to stray (GLM 7, Chi=5.9; df=3; p=0.1), however, proportionally more individuals migrated towards the larger rivers Villestrup and Kastbjerg (Table 6). There were differences in the time spent at sea between natal homers and strayers (GLM 5; Days at sea; F=10.0; df=1; p=0.002), with strayers spending less time at sea (Table 5). There were no differences in length at tagging (GLM 7; Length; Chi=0.4; df=1; p=0.5) and out-migration phenology (GLM 7; out-migration phenology; Chi=2.4; df=1; p=0.1) between strayers and natal homers.

Table 6. Number of strayers divided according to the river they were tagged in and the river they returned to. Number of fish are divided by the individuals that only returned to a given non-natal river : or both to their natal and non-natal river.

River of Tagging	River of return					
	Villestrup	Kastbjerg	Valsgaard	Maren Møllebæk	Valsgaard and Villestrup	Total
Villestrup	-	13:1	1:1	1:0	0	17
Kastbjerg	3:1	-	0	0	0	4
Valsgaard	4:6	2:1	-	6:0	0	19
Maren Møllebæk	0:3	5:2	2:0	-	1:0	13
Total	17	24	4	7	1	

Predation by cormorants

In total, 382 tags (11%) were detected across two cormorant colonies (305 and 77 in the Villestrup and Kielstrup colony, respectively) situated in Mariager Fjord. By correcting the raw tag count with the scanning efficiency (see Källo et al. 2020), it was estimated that 14.1% of the tagged individuals were predated by great cormorants, with the majority of the predated individuals (69%) not detected out-migrating, implying they were eaten in the river. The

likelihood of any tagged fish being eaten by cormorants differed among the rivers (GLM 8; River of tagging, Chi=30.3; df=3; p<0.0001), with individuals from river Valsgaard having the highest likelihood of being predated (Table 7). The patterns of predation were further investigated among individuals that out-migrated from their natal river. Out-migration phenology had an effect on predation likelihood, with autumn migrants having a higher likelihood of being eaten (GLM 9; Out-migration phenology; Chi=5.4; df=1, p=0.02, Table 7). There was no significant difference in predation likelihood across rivers among individuals that out-migrated (GLM 9; River of tagging; Chi=4.5; df=3; p=0.2).

Table 7. Number of tagged brown trout eaten by cormorants in total, subsequent to out-migration and depending whether they migrated in the autumn or spring

River of Tagging	No. of individuals predated by cormorants ^a		No. of individuals eaten subsequent to out- migration ^b		No of autumn/spring out- migrants eaten by cormorants ^b
	2018	2019	2018	2019	
Villestrup	45 (9.0%)	38 (7.6%)	12 (14.3 %)	2 (9.5 %)	6 (12.8 %) / 8 (13.8 %)
Kastbjerg	63 (12.8 %)	52 (10.4%)	12 (20.0 %)	2 (7.1%)	9 (20.0%) / 5 (11.6 %)
Valsgaard	64 (12.9%)	84 (16.8 %)	34 (20.6 %)	36 (20.3%)	55 (23.1%) / 15 (14.4 %)
Maren Møllebæk	25 (7.9 %)	11 (6.1%)	16 (17.6 %)	5 (9.3 %)	20 (16.3%) / 1 (4.5 %)
Total	197 (10.9%)	185 (11.0%)	74 (18.5%)	45 (16.1%)	90 (19.9%) / 29 (12.8 %)

^a Percentage is calculated based on a total number of individuals tagged in a given year and river

^b Percentage is calculated based on the total number of individuals that were detected out-migrating in a given year and river

Discussion

Brown trout display a continuum of life-history strategies and are known to have high phenotypic plasticity throughout their lifecycle and across their distribution range (Birnie-Gauvin et al., 2019; Ferguson et al., 2019; Klemetsen et al., 2003). In this study, intraspecific differences in life-history strategies of out-migrating juvenile and returning adults originating from four nearby rivers were investigated. Fish were tagged as juveniles in rivers that flow into the same fjord system, thus environmental conditions in the marine habitat were similar for all individuals regardless of the river of origin.

Our findings highlight differences between rivers in various life-history characteristics, at different life stages (juveniles and adults). More precisely, there were differences between the rivers in the propensity of individuals that out-migrated, with individuals originating from the two smaller rivers having a higher likelihood of out-migrating. The majority of the out-migration occurred during the autumn, with length and condition affecting which phenotype (spring or autumn) was adopted. Subsequent to out-migration, 18% of sea trout returned to freshwater, with the likelihood of returning depending on river of origin, as well as out-migration phenology. Sea trout in Mariager fjord returned to all four rivers, with 37% of individuals straying to non-natal rivers. While the river of origin did not affect the likelihood of straying, strayers returned to the two largest rivers to a higher degree.

Likelihood of out-migrating from the natal river

We detected 20% of the tagged individuals out-migrating from their natal river, though this is likely an underestimation given flooding in River Villestrup and Kastbjerg in the spring of 2020, which likely affected the estimation of out-migration rate for individuals tagged in 2019. We further documented that the likelihood for individuals to out-migrate (at least for individuals tagged in 2018) to sea was not uniform between the rivers. Juveniles from the two

smaller rivers, Valsgaard and Maren Møllebæk, were more likely to leave their natal river compared to individuals originating from the two larger rivers, Villestrup and Kastbjerg.

Several hypotheses may explain the differences in the rate of out-migration among the rivers. Firstly, there may be a difference between the rivers in the proportion of individuals opting for residency over anadromy. The decision to out-migrate or assume residency is thought to be a phenotypically plastic trait that is controlled by the environment, with genetics pre-determining the set of thresholds that underlie the decision process (known as the “threshold model”; Ferguson et al., 2019; Thorpe & Metcalfe, 1998; Wysujack et al., 2009). This means that if an individual’s condition or energetic status falls below a specific threshold at a specific time, it would out-migrate, because the conditions in the river cannot meet the demands of that fish. These thresholds can vary within and between populations (Ferguson et al., 2019) and may have been higher in Valsgaard and Maren Møllebæk, promoting anadromy. Both of these rivers are small, shallow, and may be subject to extremely low flow and partial drying out in the summer, or freezing during the winter. These are all factors that could affect the energetic state of individuals, which has been directly linked to the propensity to out-migrate, with increased out-migration occurring at low energetic states (Archer et al., 2019; Birnie-Gauvin et al., 2021; Boel et al., 2014; Wysujack et al., 2009). As such, the energetic demands of some individuals in these two streams are unlikely to be met in the river, causing them to fall below the threshold for residency, and thus adopting anadromy.

Alternatively, or concurrently, the lower out-migration rate in the two larger rivers could be associated with differences in survival between the rivers prior to out-migration. This may be related to overwintering mortality, which can be high among juvenile brown trout (Midwood et al., 2015), or predation. In this study, it was estimated that a minimum of 14% of the tagged individuals were predated by cormorants, with a high proportion of predation occurring in the river. In river Villestrup, even higher predation rates have been documented previously, with

minimum estimates of up to 34% (Källo et al., 2020), indicating that there is an overall high predation pressure of cormorants in this system. However, river Valsgaard, where the largest number of individuals were detected out-migrating, also had the highest predation rate, contradicting the importance of predation behind the documented patterns. We cannot, however, exclude that the presence of other predators, like herons and otters, which have been observed in this system (del Villar-Guerra et al., 2014), may affect the survival of juveniles prior to out-migration.

It cannot be excluded that the documented patterns of out-migration may have been affected by possible bias of tagging towards autumn migrants and residents, as it took place in July, after many spring migrants (and perhaps autumn migrants from the previous year) had already left. Thus, the lower out-migration rate in the larger rivers, may therefore be an indication that a proportion of individuals had already out-migrated in the spring or autumn prior to tagging, reducing the proportion of out-migrants among the juveniles tagged in the summer. We note that a similar bias is also present in studies tagging fish in the spring, prior to the peak juvenile out-migration period, where individuals migrating the previous autumn cannot be accounted for (Birnie-Gauvin et al., 2019). To be able to determine the presence of a sampling bias towards residents and “late” autumn migrants in this study (or towards spring migrants in other studies), aging of juveniles would be necessary, but not possible here given that scales were not collected. Regardless, we find it unlikely that this possible bias would have major consequences on our findings given the patterns found here. Moreover, there is no reason to believe that this bias did not affect all rivers equally, yet we still find strong river effects in several of the assessed metrics.

In previous studies, length, growth and condition have been used as proxies for the threshold variables that determine individual propensity to migrate, although contrasting results have been documented (Acolas et al., 2012; Birnie-Gauvin et al., 2021; Peiman et al., 2017). In the

current study, no differences in length nor condition were documented between individuals that out-migrated and those that did not. However, considering that the fate of the individuals that did not out-migrate (whether they died or assumed residency) was not determined, the differences between migrants and residents could be masked due to our inability to differentiate residents from the individuals that died. Yet, an earlier study from river Villestrup did find differences in size between migrants and non-migrants despite not determining the fate of non-migrants (Peiman et al., 2017), similarly to the present study. The two studies did differ in the timing of tagging (July vs October), which may suggest that the differences in length and condition between residents and migrants do not become evident until later in the autumn.

Out-migration phenology

As expected, out-migration from freshwater took place throughout the year, with a peak in spring and in autumn, a pattern that has been documented previously in different geographical areas (Birnie-Gauvin et al., 2021; Jonsson and Jonsson, 2009). More individuals were detected out-migrating in the autumn than in spring in all rivers except Villestrup. A lower proportion of autumn migrants has also been documented in river Villestrup in previous studies (Peiman et al., 2017; Winter et al., 2016), perhaps indicating that this is a temporally stable pattern, as variable ratios of spring to autumn migrants have been documented in other geographical areas (Marine Institute, 2014). The differences in the proportion of autumn to spring migrants documented across nearby rivers in the current study demonstrate that this pattern may already vary over short distances.

The length and condition of juveniles at the time of tagging had an effect on whether out-migration occurred in the autumn or spring. The effect of length was most pronounced in the two larger rivers, Villestrup and Kastbjerg, where shorter individuals were more likely to postpone out-migration until the following spring, while longer individuals were more likely to

out-migrate in the autumn. The effect of length was, however, not the same across rivers; in the two smaller rivers Maren Møllebæk and Valsgaard, the effect of length was significantly smaller, if not negligible. Previous work examining the effect of length on spring and autumn out-migrating sea trout generated contrasting results. Studies have documented autumn migrants being both longer (Holmes et al., 2014) and shorter (Kennedy et al., 2022) than spring migrants, while several other studies have failed to find differences in length between the phenotypes (Birnie-Gauvin and Aarestrup, 2019; Birnie-Gauvin et al., 2021), including an earlier study from river Villestrup (Winter et al., 2016). The timing of tagging did differ across studies, which may explain, at least partially, these differences.

In contrast to length, condition had a similar relationship to migration timing across all rivers; autumn migrants had lower condition factors, which is in agreement with previous findings (Birnie-Gauvin et al., 2021; Winter et al., 2016). Poorer condition among autumn migrants could indicate that the individuals could not meet their energetic needs in the river or did not have the necessary energy reserves to survive winter and thus migrated to sea early (Ferguson et al., 2019). This could mean that at the time of tagging (July), the decision to migrate in the autumn was already made. Among spring migrants, the decision to out-migrate is thought to be taken months in advance of migration (Thorpe and Metcalfe, 1998), given that physiological changes are needed to adapt for life in the marine environment. This may not be the case for autumn migrants, as there is some evidence suggesting that autumn migrants are not (fully) smoltified (Poole et al., 1996; Taal et al., 2014). Autumn migrants may therefore take the risk of out-migrating without being completely prepared for marine entry because their energetic status is poor. However, incomplete smoltification during sea entry does not automatically equal evolutionary failure, as non-smoltified individuals are able to survive and acclimate to increased salinity in the sea (Seidelin et al., 2000) and have also been documented to return in high proportion (del Villar-Guerra et al., 2019).

We found differences in the timing of out-migration between the rivers among autumn, but not spring migrants. Autumn migrants from the smallest river (Maren Møllebæk) migrated significantly earlier than autumn migrants from the other rivers. Maren Møllebæk also had the highest proportion of individuals that migrated during the autumn, perhaps indicating that conditions in that river are not suited to overwintering and/or spring migration (e.g., low food availability/high competition, thermal stress, etc.), which urges individuals to migrate at the earliest possible opportunity. In this case, autumn migration would allow fish to escape unfavorable conditions for growth or survival (i.e., fitness advantages).

Return to freshwater

We detected 18% of out-migrating individuals returning to any of the four rivers monitored in this study. Although this return rate is within the range of what has previously been documented for sea trout in other systems (Jonsson and Jonsson, 2009), it is considerably higher than previously reported by studies in the same rivers (8.3-10.0 %; del Villar-Guerra et al., 2019; Källo et al., 2022a). The mechanism for the higher return rate in the current study is unclear, but is likely due to this study following the return of individuals to both natal and non-natal rivers, as well as environmental stochasticity, which has been suggested to cause variation in return rates across years (Jonsson and Jonsson, 2009).

The likelihood to return was affected by the timing of out-migration (spring vs autumn), and differed between rivers. For example, spring migrants originating from the southern side of the fjord (Kastbjerg and Maren Møllebæk) were more likely to return than autumn migrants, while autumn migrants were more likely to return than spring migrants in river Villestrup. Individual likelihood of return was not affected by out-migration phenology in river Valsgaard. Contrasting results in the return rate of autumn migrants have been recorded. For example, Jonsson and Jonsson (2009) documented lower return rates among autumn migrants in river

Imsa, Norway, while Birnie-Gauvin and Aarestrup (2019) found no differences between autumn and spring migrants in river Gudsø, Denmark. The results from this study highlight that even individuals that originate from rivers in close proximity, and which share the same marine environment, can show markedly different return rates between autumn and spring migrants. The reasons for these differences are unclear, but there are some indications that spatiotemporal differences in predation regime may have an effect. We found that autumn migrants were more likely to be eaten by cormorants, which may, in part, explain why autumn migrants had a lower return likelihood in two of the rivers. This is supported by the fact that in River Villestrup, the only river where autumn migrants had a higher likelihood of return, had the smallest difference in predation likelihood between spring and autumn migrants. We acknowledge that we found no statistically significant effect of river of tagging on predation likelihood for spring and autumn migrants, but the notably small difference in predation between the two phenologies in River Villestrup may indicate a pattern that we cannot elucidate due to small sample sizes. It further, cannot be excluded that the differences in return rate between spring and autumn out-migrants stem from other factors affecting individual survival at sea, like differences in behavior (e.g., habitat utilization), and distribution of predators, other than cormorants (Strøm et al., 2019; Ward et al., 2012). Neither length nor condition – factors that have been documented to affect return probability among salmonids (Armstrong et al., 2018; del Villar-Guerra et al., 2019) – were documented to have an effect of the likelihood of return in the current study.

Patterns of straying

Of all returning sea trout, 37% were detected in non-natal rivers, with the majority (66%) being in the non-natal river during the spawning season, likely suggesting they were there to spawn. Straying in salmonids has been qualitatively documented using genetic methods (King et al.,

2016; Massa-Gallucci et al., 2010), but quantitative estimates are scarce. Källo et al. (2022a, b) has previously investigated straying in the Mariager Fjord system using tagged spring migrating individuals originating from river Villestrup, and estimated that at minimum, 37% of tagged fish strayed to other rivers in the fjord. In this study, straying patterns were explored using fish tagged in river Villestrup, as well as three additional rivers to investigate whether straying occurred in a similar proportion in all the rivers. Our findings suggest this is the case, as no significant differences in straying rates were found among rivers. This compliments our previous findings (Källo et al., 2022a,b) by demonstrating that straying is a common strategy in sea trout from Mariager fjord. Although we found no clear pattern of straying based on the river of origin, straying to the larger rivers (Villestrup and Kastbjerg) seemed to be more prominent. This is not particularly surprising, as straying from a smaller natal river to a larger non-natal river has been previously documented among salmonids (Degerman et al., 2012; Quinn, 1993;). We further documented that the likelihood of straying was dependent on the time spent at sea, with strayers tending to have shorter marine phases than natal homers, which has also been similarly documented in river Villestrup previously (Källo et al., 2022a). The sea is a dangerous environment (Kristensen et al., 2019; Källo et al., 2022a), and strayers may adopt a sort of bet-hedging strategy where they return to freshwater after a shorter period at sea to spawn at a smaller size. In this way, strayers ensure a lifetime fitness above zero and avoid evolutionary failure. Our data cannot support or refute this hypothesis since the reproductive output (and lifetime fitness) of strayers and natal homers was not assessed in this study. However, this would represent a crucial next step if we are to fully understand the importance of straying as a strategy in salmonids.

Perspectives and conclusions:

Sea trout display a continuum of life-history strategies, and do so throughout their lifecycle. In this study, we contribute to this literature by showing that juveniles out-migrate both in autumn and spring, with the frequency of these strategies differing between rivers. The timing of autumn migration also varies, perhaps depending on river-specific conditions that affect fitness. It appears that opting for the decision to out-migrate in autumn or spring can be an important one, as return rates differed significantly between the two phenotypes, depending on river of origin. Our findings highlight that disregarding autumn migrants in estimates of out-migration means disregarding a part of the population that likely contributes to recruitment, which may be more significant in some rivers than others. We further show that returning sea trout vary in the duration of their marine phase, which tended to be shorter for those that strayed. Furthermore, we observed straying in fish originating from all four rivers, perhaps suggesting that straying is an alternative strategy to homing in this fjord. The latter combined with the finding that 37% of returning sea trout strayed, with most straying to larger rivers, supports the idea that straying in this system is likely not a maladaptive ‘error’, but rather a strategy that may confer fitness related benefits for some individuals.

By following the migratory behavior of brown trout from four donor and recipient populations that share the same marine system, we contribute to our understanding of two major knowledge gaps in salmonid biology: that of autumn migration and straying. We documented a wide variety of life-history strategies, but highlight the high prevalence of autumn migration and straying in all four studied rivers. To further deduce the drivers of these strategies, how they may differ among rivers, as well as the fitness related costs and benefits associated with them, a natural next step would be to sex and age tagged individuals. Doing so would provide us with additional information on, for example, whether males or females preferentially adopt one strategy over the other, which may further shed light on the ultimate mechanisms at play here.

Monitoring the fate of individuals that did not out-migrate (i.e., whether they assumed residency or died in the river) would also provide us with a more holistic understanding of life-history strategies. Although the drivers of movement strategies studied here remain uncertain, our study is the first to assess both spring vs. autumn migration and homing vs. straying, contributing to our understanding of two largely overlooked aspects of salmonid biology and providing important insight into intraspecific life-history variability.

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Conflict of interests:

Authors have no conflict of interests to declare

Author Contribution:

KBG and KA contributed to the conception and the design of the study. KBG carried out the fieldwork. KK and HB contributed to handling and analysing of the data. All authors contributed significantly to the interpretation of the results and have given their final approval for the manuscript to be published.

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Data will be made available by the authors upon reasonable request

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MANUSCRIPT IV: Otolith microchemistry combined with genetics reveal patterns of straying and population connectivity in anadromous brown trout (*Salmo trutta*)

Submitted

Otolith microchemistry combined with genetics reveal patterns of straying and population connectivity in anadromous brown trout (*Salmo trutta*)

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Abstract:

Salmonids are well known for their natal homing behaviour, meaning they return to breed in the same area where they originated. However, not all individuals return to their natal breeding grounds – a behavioural trait known as straying. The prevalence of straying is difficult to explore and therefore quantitative estimates for straying are seldom reported. In this study, otolith microchemistry and genetics were combined to investigate patterns of straying over ecological and evolutionary time, respectively, between neighbouring rivers flowing into Mariager fjord, Denmark. Otolith microchemistry was used to determine the river of origin for sea trout (*Salmo trutta*) upon their return to freshwater and 288 SNP markers were used to estimate the level of gene flow between the rivers in the fjord. In this system, where the distance between rivers is short, otolith microchemistry achieved 80% accuracy in assigning juvenile brown trout to their natal river, thus allowing us to determine that approximately 43% of the adult sea trout had returned to non-natal rivers to spawn, with similar proportion of strayers and natal homers in all of the rivers. Genetic analysis further showed that there was a high level of gene flow among individuals originating from different rivers, indicating that sea trout in Mariager fjord make up one population. The findings obtained from otolith microchemistry and genetics complement each other and provide further evidence that sea trout in this system migrate to non-natal rivers and spawn there, which consequently affects the genetic structure of the population.

Key words: gene flow, natal homing, dispersal, salmonids, phenotypic plasticity,

Introduction

The movement of fish between populations may have varying levels of impact on both the donor and recipient population by affecting population dynamics, demographics and genetic structure (Bett et al., 2017; Bowler & Benton, 2005; Schtickzelle & Quinn, 2007). Therefore, assessing population connectivity and structure by tracking individuals' movements as well as determining genetic differentiation among populations over evolutionary timescales is essential to understand the factors that affect population sustainability. This is especially relevant in the Anthropocene, when many populations are increasingly vulnerable to negative effects caused by climate- and habitat changes and other anthropogenic factors (Last et al., 2011).

Otolith microchemistry and genetics have proven to be effective tools for investigating population structure and connectivity in fishes (Bekkevold et al., 2020; Collins et al., 2013; Heidemann et al., 2012). Otolith microchemistry provides the possibility to infer information on migratory patterns (Brennan et al., 2015; Sturrock et al., 2015b; Taal et al., 2014;), habitat use (Ciepiela & Walters, 2019; Phillis et al., 2018; Volk et al., 2010), and the origin of fish among marine, freshwater, and diadromous populations (Matetski et al., 2022; Chen et al., 2020; Heidemann et al., 2012). Otoliths are particularly well suited to track migrations among aquatic ecosystems, as they grow continuously and incorporate chemical differences among water sources over the lifetime of an individual (Campana, 1999; Svedäng et al., 2010; Tabouret et al., 2010).

The capability of otolith microchemistry to discriminate populations is contingent on the assumption that individuals that originate from different habitats differ in their chemical composition of the otolith (Brennan et al., 2015; Campana et al., 1994; Chang & Geffen, 2013). While the physio-chemical properties of water affect otolith microchemistry (Brown & Severin, 2009; Macdonald & Crook, 2010), other factors, for example physiology (Sturrock et

al., 2015a) and ontogeny (Walther et al., 2010) are also involved. Therefore, the extent to which otolith microchemistry differs between populations vary among different species and systems (Chang & Geffen, 2013), resulting in varying levels of discrimination (Collins et al., 2013; Morales-Nin et al., 2022; Turcotte & Shrimpton, 2020).

While otolith microchemistry allows us to follow the movements of individuals over their life cycle, genetic marker analysis ('genetics') - a central tool in studies of population connectivity - offers the opportunity to disentangle patterns of population structure over an evolutionary time (Bekkevold et al., 2020; Massa-Gallucci et al., 2010; Waples & Gaggiotti, 2006). Although, genetics can also be applied to identify immigrants from demographically isolated populations (Bekkevold et al., 2004; Masson et al., 2018), in species that are characterised by larger population sizes and high dispersal, identifying immigrants is often not possible due to weak genetic differentiation among populations. Nevertheless, genetics provide valuable insights into evolutionary forces, including population size and patterns of selection, which may affect population structure. Consequently, combining both otolith microchemistry and genetics through an interdisciplinary approach could provide resolution for population connectivity and structuring over varying timescales.

Salmonids are well known for their natal homing behaviour, meaning they return to spawn in the same area where they hatched (Jonsson & Jonsson, 2014; Keefer & Caudill, 2014). It is an evolutionarily important mechanism through which individuals increase their likelihood of finding suitable habitats and mates during the breeding season (Keefer & Caudill, 2014). It is also a mechanism through which inter-population genetic structuring takes place, and local adaptations develop (Hendry et al., 2004; Peterson et al., 2014). Consequently, in salmonids the spatial-scale of population boundaries are often defined as the rivers or tributaries individuals originate from or return to spawn, because dispersal and overall connectivity among rivers is considered to be low (see Miettinen et al., 2021 and references herein). The prevalence

of natal homing may however vary significantly between species of salmonids, as well as between populations of the same species (Ayllon et al., 2006; Östergren et al., 2012; Quinn, 1993). For example, among anadromous brown trout (*Salmo trutta*), also referred to as sea trout, straying rates have been previously documented to vary between 1.6 and 55% among wild individuals (Jonsson & Jonsson, 2014; Källo et al., 2023a). However, quantitative estimates on the rate of straying are scarce, limiting a complete assessment of the prevalence of straying in different geographical regions, and its potential impacts on population dynamics. In Mariager fjord, Denmark, significant levels of straying (12-55%) have been previously documented among sea trout originating from four different rivers using Passive Integrated Transponder (PIT) telemetry (Källo et al., 2022a, 2022b, 2023a). However, as telemetry is not a feasible solution in many systems, the aim of this study was to test whether otolith microchemistry and genetic methods can be used to assess population connectivity (via straying and/or gene flow) among anadromous brown trout originating from rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk flowing into the same marine system. More precisely, otolith microchemistry was used to assign spawning adult sea trout to their river of origin, thus quantifying the number of spawners that had strayed and genetic analysis (SNP analyses) was used to infer information on gene flow among the rivers in the fjord and determine patterns of reproductively successful straying over an evolutionary time.

Methods

Study area

Juvenile and adult brown trout were sampled from four rivers: Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk. All rivers flow into Mariager fjord, Denmark, which is situated in Northern Jutland, and flows to the Kattegat (Figure 1). The fjord is about 40 km long, 2 km wide and has a maximum depth of 30 m. The surface water salinity varies from around 12 ‰ in the inner part of the fjord, to >20 ‰ in the outer part of the fjord (Fallesen et al., 2000).

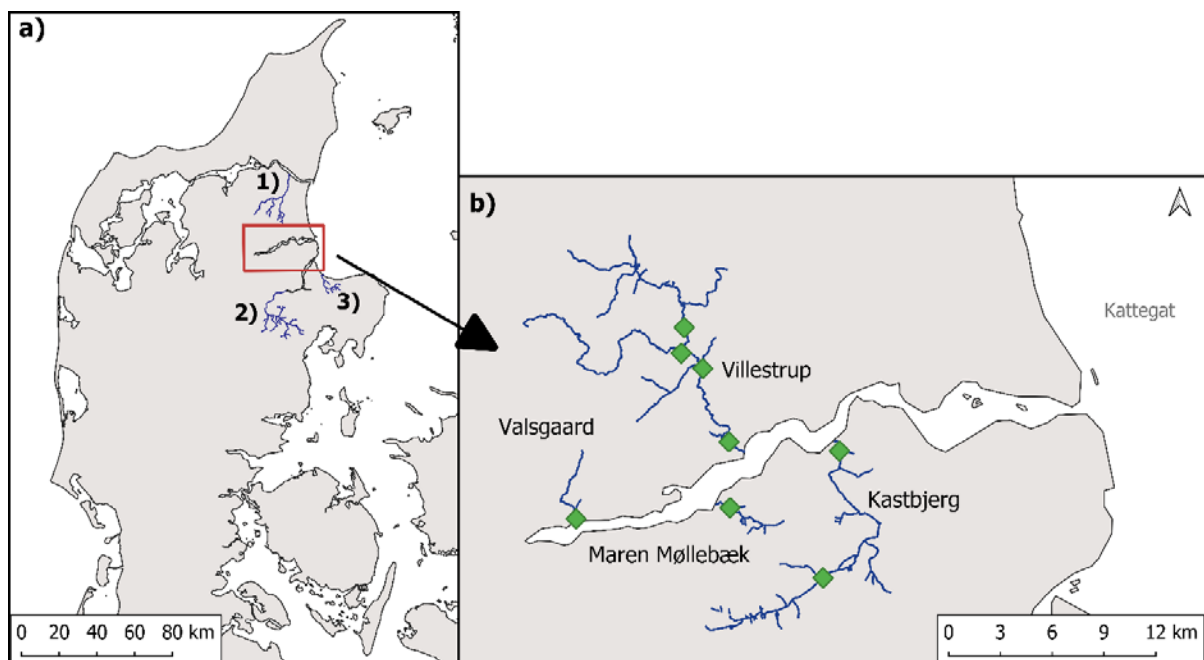


Figure 1. Juvenile and adult brown trout were collected from Rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk (b) to identify strayers and determine gene flow among the rivers. We further determined gene flow between individuals from the rivers of Mariager fjord and the neighboring rivers Lindenborg (1), Lilleaa (2), and Hevring (3), which are situated outside of Mariager fjord (a). Green diamonds mark the sampling stations where juvenile brown trout were collected in 2020 and 2021 (b).

Sample collection and preparation for microchemistry analysis

Juvenile brown trout were collected in March 2020 and 2021 via electrofishing, at one or multiple stations in each river (Figure 1), with approximately 20 individuals randomly collected

at each location per year (Table 1). After being caught, juvenile brown trout were immediately euthanized via an overdose of benzocaine (Sigma Chemical Co., St Louis, USA). All juveniles were of a size consistent with fish that have never been to sea (Table 1), so we assume these fish had hatched in the river of capture. Adult sea trout were caught via electrofishing each of the four rivers during the spawning season in November-December 2020 and immediately euthanized upon capture through a blow to the head. The sampling scheme for adult sea trout aimed for a 1:1 sex ratio, which was roughly achieved for all of the rivers, besides Møllebæk, where too few males were caught.

Total length was measured for all fish (to the closest cm) and adult sea trout gonads were visually inspected to ensure maturity. All trout were sampled for genetic analysis, by cutting the adipose fin and storing it in 96% ethanol. Otolith pairs (*sagitta*) were removed for all trout with tweezers, cleaned, dried and stored in Eppendorf vials. Prior to otolith microchemistry analysis, one otolith per fish was chosen at random, embedded in two-part epoxy (Epofix; Struers), and ground in transversal plane using abrasive papers with grit size P800-P2400 (Struers) until the core of the otolith was visible. All otoliths were polished with abrasive paper of grit size p4000 (Struers), and finally glued to a glass slide using superglue.

Otolith microchemistry analysis

Otolith microchemistry analysis for trace elements was conducted at the department of Geoscience at Aarhus University by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) using a Resonetics 193 nm laser coupled to an Agilent 7900 Quadrupole instrument. Concentrations of ^{88}Sr , ^{43}Ca , ^{55}Mn , ^{24}Mg , ^{66}Zn , ^{138}Ba and ^{208}Pb were measured through a transect of consecutive spots of 60 μm in diameter along the longest growth axis of the otolith with laser energy set at 80 mJ, pulse frequency at 10 Hz and 90 seconds data acquisition times. Background levels were measured for 30 seconds before and after each spot

analysis. Juvenile otolith transects were set from the edge of the primordium until the edge of the otoliths. Adult otolith transects were set from the edge of the primordium until the presumed freshwater exit, based on visual inspection of growth bands. LA-ICPMS data was processed with the open-source Python package *LAtools* (Branson et al., 2019). The software automatically removed instrument artifacts (despiking), subtracted background, normalized raw intensities to the internal standard (^{43}Ca) and computed element/Ca ratios in mol/mol for samples using two-point calibration curves based on NIST612 and NIST610 glasses analyzed during the run. NIST standards were analyzed every three to four otoliths to monitor for instrumental drift. To determine the river of origin of adult sea trout, the otolith section containing the freshwater stage was determined, based on a significant increase in Sr/Ca and concurrent decrease in Ba/Ca values, along the otolith microchemistry transect (Macdonald & Crook, 2010).

In addition to trace element analysis, strontium isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) were measured at the University of California-Davis Interdisciplinary Center for Plasma Mass Spectrometry, using a solid state Nd:YAG 213-nm laser (Elemental Scientific Lasers, UP213) coupled to a (NuAmetek) Plasma HR Multiple-Collection Inductively Coupled Plasma Mass Spectrometer (LA-MC-ICP-MS), following established protocols (Willmes et al., 2021). Briefly, all otoliths were ablated from the edge of the primordium until the ventral edge (juveniles) or presumed first freshwater exit (adults), using a beam diameter of 40 μm , moving 5 $\mu\text{m}/\text{s}$, at 10 Hz frequency and a fluence of 4-6 J/cm^2 . Primary data handling was conducted using the *IsoFishR* package for R (Willmes et al., 2018), with which we applied a normalization for mass bias, ^{87}Rb interference correction, and on-peak subtraction for ^{86}Kr . Outliers were removed based on a 20-point moving interquartile range (IQR) criterion. Accuracy and reproducibility of the LA-MC-ICP-MS were evaluated using an otolith isotopic reference material from a white seabass (*Atractoscion nobilis*) collected offshore of Baja California, which yielded a mean

$^{87}\text{Sr}/^{86}\text{Sr}$ value of 0.70912 ± 0.00013 ($n=72 \pm 2\sigma$) in good agreement with the global average $^{87}\text{Sr}/^{86}\text{Sr}$ value of modern seawater of 0.70918 (Veizer et al., 1999). For all otoliths, element/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were averaged over the freshwater phase of each individual.

Genetic marker analyses

A subsample of juveniles chosen for otolith microchemistry analysis were selected for genetic analysis (Table 1) to investigate genetic structuring among the rivers in Mariager fjord and in comparison with the adjacent rivers outside of the fjord (Figure 1). To further examine the temporal stability of genotype data, we extracted data for 34 adult sea trout sampled from Villestrup in 2011, which corresponds to approximately three generations prior to those sampled here. Temporal data was not available for the other three rivers within Mariager fjord. Genetic analyses of juvenile trout sampled in this study were based on the analysis of 288 SNP markers selected from a genome-wide SNP panel analyzed for trout populations spanning large parts of Northern Europe (Bekkevold et al., 2020). The 288 SNPs were selected to maximize resolution among local Danish trout populations (Bekkevold et al. unpublished). DNA was extracted from fin clips and genotyping was performed using methods described in Bekkevold et al. (2021).

Table 1. Sample sizes and total lengths (cm) of juvenile brown trout collected in rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk for otolith microchemistry and genetics analysis, divided by river and year of sampling.

River	Number of juveniles included in otolith microchemistry analysis (2020/2021)	Number of juveniles included in the genetic analysis (2020/2021)	Length \pm SD of juveniles collected for analysis
Villestrup	74 (53/21)	30 (10/20)	9.4 \pm 2.3
Kastbjerg	55 (33/22)	30 (10/20)	10.9 \pm 2.6
Valsgaard	34 (20/14)	20 (10/10)	9.4 \pm 2.2
Maren Møllebæk	30 (15/15)	24 (10/14)	9.7 \pm 3.3
Total	193 (121/72)	104 (40/64)	9.9 \pm 2.6

Otolith microchemistry data analysis

A Random Forest (RF) supervised machine learning algorithm, which has shown to provide high classification accuracy in similar studies (Mercier et al., 2011), was used to determine the origin of adult sea trout caught in rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk based on otolith microchemistry data. Using package *randomForest* for R (Liaw and Wiener, 2002), a RF classifier was built using the juvenile otolith microchemistry data, and was subsequently used to assign spawning adult sea trout, whose origin was unknown, to their river of origin. The juvenile otolith microchemistry data was divided into training (75%) and validation data (25%), where the former was used to build the classifier and the latter to determine overall classification accuracy (\pm SD) of the classifier and for each of the rivers. Each tree in the forest was constructed using a bootstrap aggregated dataset where at each node, a random subset of elemental ratios was used to find the largest discrepancy between the groups. At each node, three random variables were selected, and in total 400 trees were constructed. To determine the deviance of the RF classifier, 500 RF classifiers, which used a

random subsample of the training dataset, were built and subsequently tested on randomly subsampled validation dataset. All possible elemental combinations were tested using previously described RF classifier training and validation processes, with the elemental combination producing the highest classification accuracy chosen as the final model. The final elemental ratios used in the random forest classifier that produced the lowest misclassification rate were: $^{87}\text{Sr}/^{86}\text{Sr}$, Sr/Ca, Ba/Ca, Mg/Ca and Mn/Ca.

To further determine how elemental ratios differ between rivers and years, element-specific Generalized Linear models (GLM) were applied on the juvenile otolith microchemistry dataset including the main effect of the variables. Each elemental model consisted of element/Ca or $^{87}\text{Sr}/^{86}\text{Sr}$ against river and year. This was followed by pair-wise Tukey *posthoc* comparisons to determine which rivers differed from each other based on element/Ca or $^{87}\text{Sr}/^{86}\text{Sr}$ ratios.

Each RF classifier built ($n = 500$) was used to assign adult sea trout to their river of origin (i.e., each individual was assigned 500 times), allowing us to determine the within-individual variance in the assignment to their natal river, as well as the robustness of the results, where adult assignment data was subsequently used. Adult sea trout that were assigned to originate from a river other than the one they were collected in were categorized as strayers. To investigate whether there were differences in the proportion of strayers and natal homers among adults collected in each of the rivers, a Chi squared test was applied to each assignment dataset produced by the RF classifier. To investigate the effect of length, sex, river of origin and all possible two-way interactions on the likelihood of being a strayer, a Bernoulli-distributed GLM was used on each generated assignment dataset. To determine the importance of the interactions, the original models including all main affects and interactions were compared to a model where each of the interaction was removed one at a time. A ΔAIC below -2 was used as threshold for considering an interaction as important.

Genetic data analysis

Genetic data was analysed to estimate sample-specific genetic differentiation among rivers in and outside of the fjord. As the inclusion of siblings in analyses can bias genetic estimates (Hansen et al., 1997), functions from the *Demerelate* R package (Kraemer & Gerlach, 2017) were applied to genotype data, and the relatedness between individuals was estimated using the estimator from Wang (2002). Pairs of individuals showing relatedness coefficients > 0.4 were filtered to only include a single individual, with the expectation that this would filter out full siblings (see caveats in Wang, 2014). Pairwise estimates of genetic differentiation (Weir & Cockerham's F_{st} estimator) were generated for all collections (Table 1) with the *genepop* R package (Rousset, 2008) and exact tests were used to test for statistical significance. River specific collections in 2020 and 2021 were initially analysed individually, but pooled in subsequent analyses when exact tests indicated lack of differentiation between the years. Pairwise p-values were corrected for multiple comparisons with *false discovery rate* (FDR) (Benjamini and Hochberg, 1995).

Results:

Determining a baseline otolith microchemistry fingerprint for each of the rivers

In total, 193 juvenile brown trout otoliths (Table 1), collected over two years, were analysed to obtain river-specific otolith microchemistry fingerprints, which were subsequently used to determine the origin of spawning adult sea trout caught in the same four rivers. The average classification accuracy from the RF classifier based on juvenile otolith fingerprints was $80.4 \pm 5.4\%$ (Kappa coefficient of 72.4%), with the highest classification accuracies in rivers Valsgaard (94.6%) and Villestrup (87.8%), which are both located on the northern side of the fjord (Table 2). Most notably, 21.4% of individuals from river Kastbjerg were misclassified to originate from river Villestrup, while misclassification among the other rivers was significantly

lower, between 0 and 10.0% (Table 2). The most important elemental ratios used to discriminate between the rivers were $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr/Ca , with Mn/Ca , Ba/Ca and Mg/Ca contributing less. Other elements did not contribute or had negative effects on the accuracy of the RF classifier, which resulted in those elements being removed from the analysis.

Table 2. Cross validation matrix of the random forest models indicating the level of incorrect classification \pm SD (%), with exception of bold entries in diagonal, which represent the correct classification of juvenile brown trout to their natal river.

Known river of origin	RF-classified river of origin			
	Villestrup	Kastbjerg	Valsgaard	Maren Møllebæk
Villestrup	87.8 \pm 7.7	10.0 \pm 7.2	0	2.2 \pm 3.2
Kastbjerg	21.4 \pm 10.9	65.6 \pm 12.9	7.2 \pm 6.5	5.7 \pm 6.2
Valsgaard	0	3.5 \pm 7.6	94.6 \pm 8.9	1.9 \pm 4.9
Maren Møllebæk	9.0 \pm 10.3	6.7 \pm 9.1	9.7 \pm 9.9	74.7 \pm 15.2

The element/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios included in the random forest classification tree were further analysed to investigate potential differences between rivers and years (Table 3). $^{87}\text{Sr}/^{86}\text{Sr}$ (Figure 2) and Sr/Ca differed significantly among rivers and years, Ba/Ca and Mn/Ca differed significantly among rivers, and Mg/Ca differed only between years (Table 3). $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr/Ca , which contributed the most to assigning individuals to their river of origin, differed in all pair-wise comparisons, apart from Sr/Ca between rivers Villestrup and Kastbjerg (Figure 3). Ba/Ca differed among all the rivers except for Villestrup and Maren Møllebæk; Mn/Ca differed only among Kastbjerg and Maren Møllebæk, and Kastbjerg and Villestrup.

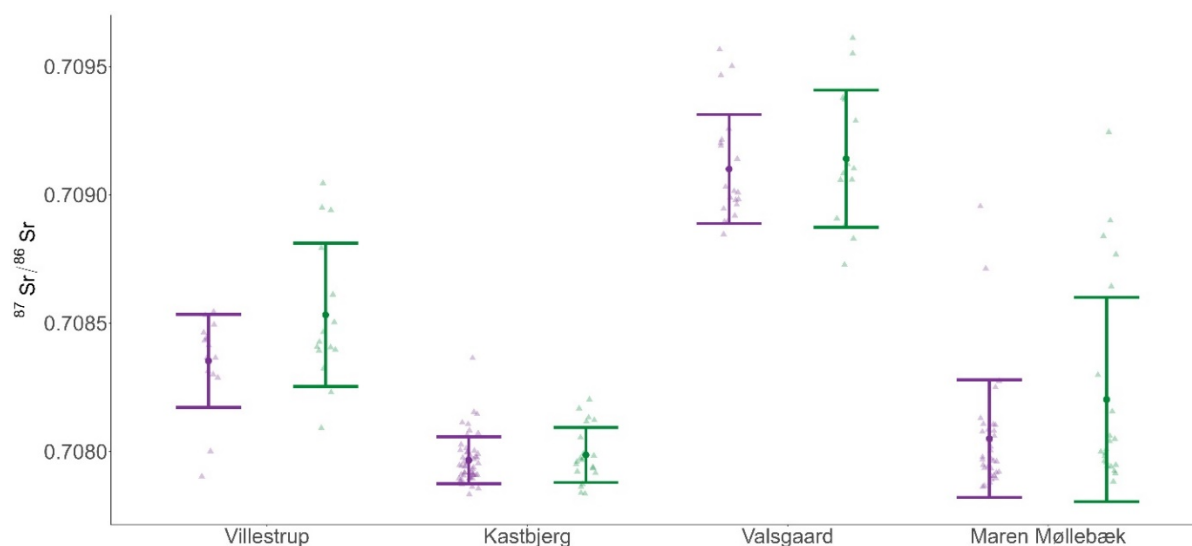


Figure 2. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios measured from juvenile brown trout otoliths collected from different rivers flowing into Mariager fjord, in 2020 (purple) and 2021 (green). Points represent mean values, whiskers standard deviation and triangles measured $^{87}\text{Sr}/^{86}\text{Sr}$ values.

Table 3. Result from the GLM analysis investigating differences in elemental ratios included in the RF classifier between rivers and years.

Element	Effect of river			Effect of year		
	df	F-statistic	p-value	df	F-statistic	p-value
$^{87}\text{Sr}/^{86}\text{Sr}$	3	225.9	<0.001	1	7.4	0.007
Sr/Ca	3	106.3	<0.001	1	7.3	0.007
Ba/Ca*	3	12.3	<0.001	1	0.1	0.7
Mg/Ca*	3	2.2	0.09	1	5.3	0.02
Mn/Ca*	3	5.8	<0.001	1	0.8	0.3

* These elements were log-transformed prior to the analysis to meet model assumption of normality.

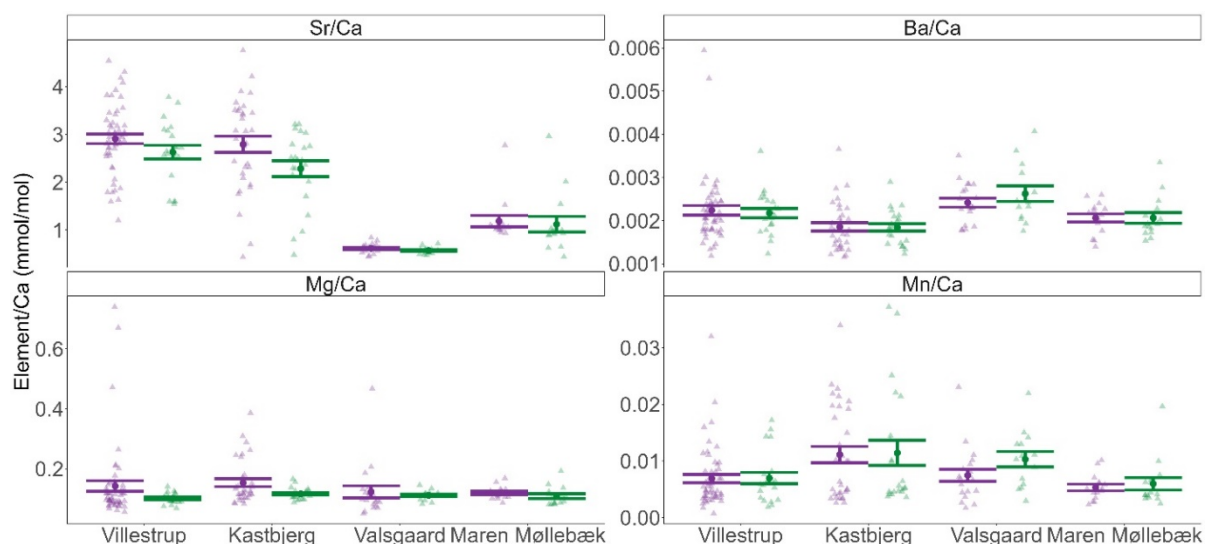


Figure 3. Element/Ca ratios measured from juvenile brown trout otoliths collected from different rivers flowing into Mariager fjord, in 2020 (purple) and 2021 (green). Points represent mean values, whiskers standard deviation and triangles measured element/Ca values.

Origin of adult sea trout and potential factors connected to patterns of straying

In total, 90 adult sea trout were collected from rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk, and assigned to their river of origin using the RF classifier (Table 4). On average, 43 ± 2 % ($n=39 \pm 2$) of individuals were assigned to originate from a river other than the one they were collected in during spawning; meaning, they are estimated to have strayed (Table 5). There were no differences between the ratio of strayers and natal homers in each of the rivers (Figure S1), with the average proportion of strayers ranging between 36% and 55% (Table 4). Proportionally, a larger number of adult sea trout had strayed to the larger rivers, Villestrup and Kastbjerg, compared to Valsgaard and Maren Møllebæk (Figure 4).

Table 4. Number of adult sea trout sampled in each of the rivers (grouped by sex), their length (min-max) and the number of individuals assigned to have strayed from/to a given river.

River	Number of fish sampled (male/female)	Average length in cm (min-max)	Number of sea trout assigned as strayers \pm SD (%; Male/female) *	Total number of sea trout \pm SD (%) assigned to have originated from a given river**	Total number of strayers \pm SD (%) assigned to have originated from a given river ***
Villestrup	22 (13/9)	59 (51-72)	8 \pm 1 (36 %; 4/4)	31 \pm 3 (34 %)	17 \pm 2 (44 %)
Kastbjerg	28 (15/13)	49 (32-64)	11 \pm 2 (39 %; 4/7)	30 \pm 3 (33 %)	13 \pm 3 (33 %)
Valsgaard	20 (10/10)	38 (26-51)	9 \pm 1 (45 %; 5/4)	19 \pm 1 (21 %)	8 \pm 0 (20 %)
Maren Møllebæk	20 (5/15)	45 (28-61)	11 \pm 1 (55 %; 3/8)	10 \pm 2 (11 %)	1 \pm 2 (3 %)
Total	90 (43/47)	48 (26-72)	39 \pm 2 (43 %; 16/23)	90	39

*Percentage is calculated based on the number of fish collected in the given river

** Percentage is calculated based on the total number of adults sampled

***Percentage is calculated based on the total number of strayer

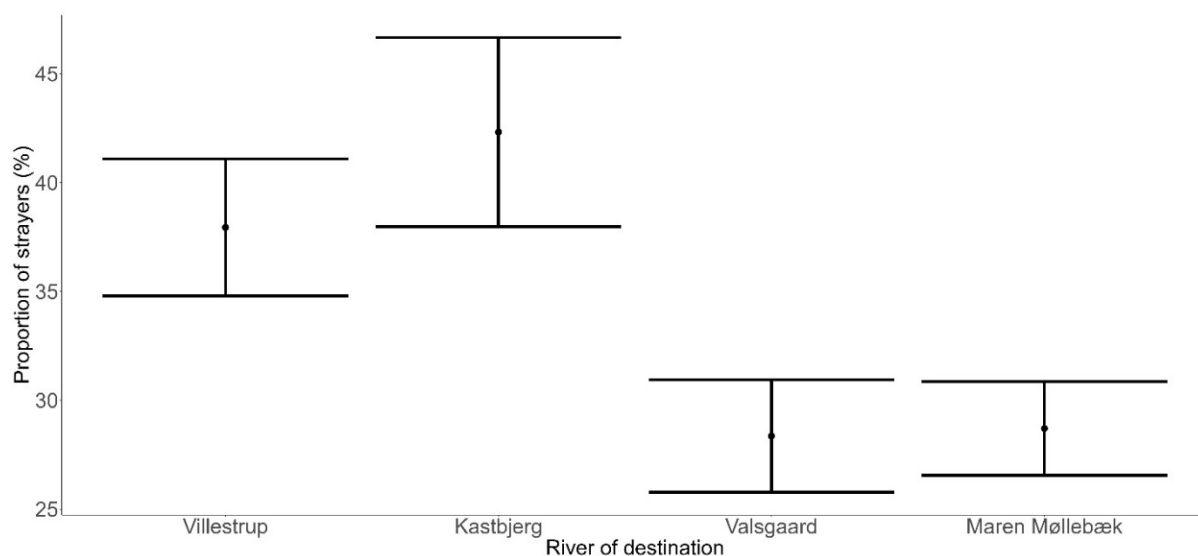


Figure 4. Estimated proportion of strayers (%) based on each of the assignment datasets (n=500) depending on whether strayers migrated to rivers Villestrup, Kastbjerg, Valsgaard and Maren Møllebæk, independent where they had originated from. The point and the whiskers represent the mean and the standard deviation, respectively.

Several factors were linked to individual's likelihood to stray. There was a strong indication that length of adult sea trout upon return to freshwater was linked to individual likelihood of straying, although the patterns varied among rivers. In all assignment datasets, excluding the interaction between individual length and river of origin produced models where the ΔAIC was substantially lower than -2, which is a strong indication for the importance of the interaction between the aforementioned variables (Figure 5). The likelihood of straying decreased with individual length in river Villestrup, but increased with individual length in rivers Kastbjerg and Valsgaard (Figure 6). Maren Møllebæk was excluded from the analysis due to the low number of individuals assigned as strayers originating from the river (Table 5).

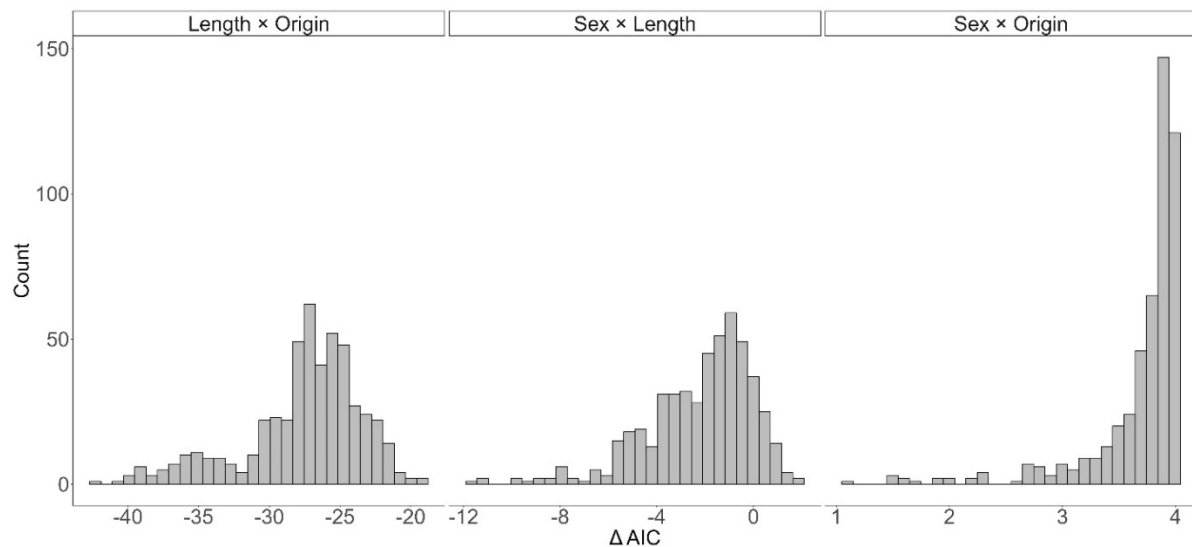


Figure 5. Δ AIC values comparing the original GLM model, containing the effects of length, river of origin, sex, and all possible two-way interactions on the likelihood of straying, to the same model excluding one of the interactions. Each comparison was performed for each of the 500 assignment datasets. A Δ AIC below -2 indicates an interaction that is linked to the likelihood of straying.

There was also some indication for the interaction between sex and length being linked to likelihood of straying, since 43% of the assignment datasets produced a Δ AIC lower than -2. However, this relationship may, at least partly, be driven by the misclassification of individuals originating from river Villestrup to river Kastbjerg (Figure S2). Further, even among RF assignment datasets, where the interaction between sex and length was considered to be important, the wide overlapping confidence intervals suggest at most inconclusive results (Figure S2). The interaction of sex and river of origin was not linked to individual likelihood of straying (Figure 5).

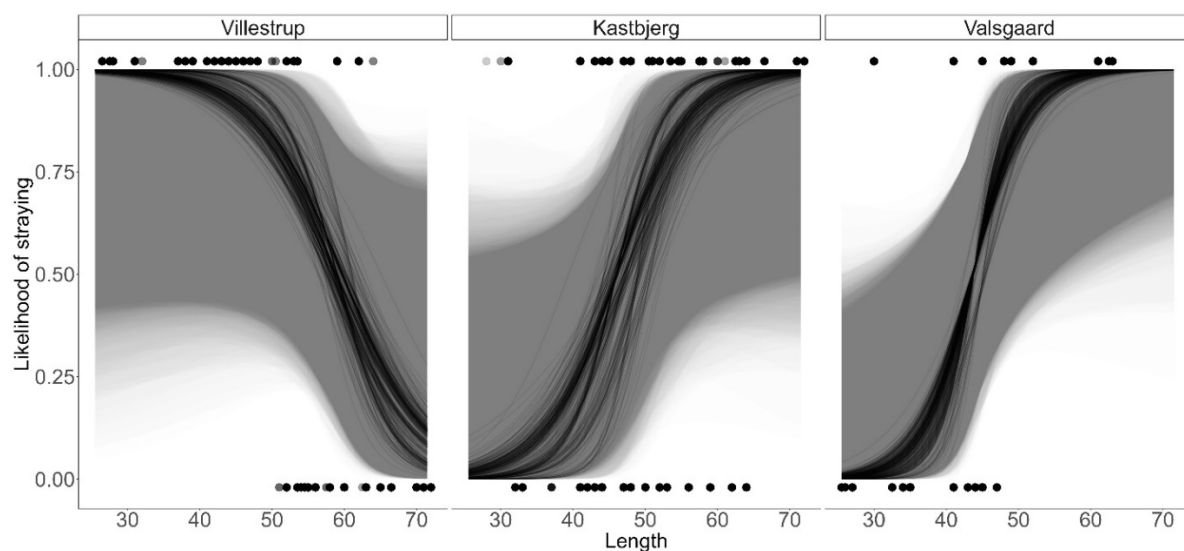


Figure 6. Modelled output of the likelihood of straying for spawning sea trout against length at capture, divided by river of origin. Individual likelihood of straying was dependent of length, with the patterns differing between the rivers. Maren Møllebæk was excluded from the analysis due to a low sample size. The lines and the shaded area represent the mean and 95% confidence intervals for each of the models. Model is conditional on sex = female. Points represent individual sea trout from each of the assignment datasets depending on whether they were assigned as strayers (“1”) or natal homers (“0”)

Table 5. Average number \pm SD of mature adult sea trout based on their assigned river of origin (rows) and the river of destination (columns), along with the total number of individuals assigned to either group.

River of origin	River of destination				
	Villestrup	Kastbjerg	Valsgaard	Maren Møllebæk	Total
Villestrup	13.8 \pm 1	10 .0 \pm 2	3.8 \pm 2	3.6 \pm 1	31
Kastbjerg	7.0 \pm 1	17.0 \pm 2	4.6 \pm 1	1.6 \pm 1	30
Valsgaard	1.0 \pm 0	1.0 \pm 0	11.2 \pm 1	5.8 \pm 0	19
Maren Møllebæk	0.1 \pm 0	0	0.5 \pm 1	9.0 \pm 1	10
Total	22	28	20	20	

Genetic estimates of differentiation

Tests for related individuals within the samples identified two pairs of fish from Maren Møllebæk with relatedness > 0.4 , resulting in the removal of one of the individuals from each of the pairs (in total $n=2$) from further analyses. No other collection showed evidence of closely related individuals. Based on tests for population differentiation, all Mariager Fjord collections showed statistically significant differentiation from all neighboring populations (Hevring, Lilleaa, Lindenberg), and no differentiation among collections within Mariager Fjord (Table 6). This is indicative of prevalent gene flow among rivers within Mariager Fjord, and restricted exchange between Mariager Fjord and neighboring rivers outside of the fjord. Comparing samples from Villestrup collected approximately ten years apart also showed a lack of genetic differentiation, although a single within-fjord comparison (Maren-Møllebæk and Villestrup 2011) showed weak, but statistically significant, differentiation at $p < 0.05$ (Table 6).

Table 6. Pairwise F_{st} estimates (below diagonal) and FDR corrected p-values for differentiation among collections (above diagonal). Values in bold represent comparisons between samples within Mariager Fjord.

	Kastbjerg	Maren-Møllebæk	Valsgaard	Villestrup	Villestrup 2011	Hevring	Lilleå	Lindenborg
Kastbjerg		0.996	0.996	0.996	0.996	<0.0001***	<0.0001***	0.0187*
Maren-Møllebæk	0.004		0.996	0.583	0.017*	<0.0001***	<0.0001***	<0.0001***
Valsgaard	0.001	0.003		0.996	0.996	<0.0001***	<0.0001***	0.0072**
Villestrup	0.001	0.006	0.001		0.916	<0.0001***	<0.0001***	0.0001***
Villestrup 2011	0.002	0.009	0.003	0.002		<0.0001***	<0.0001***	<0.0001***
Hevring	0.032	0.036	0.036	0.028	0.030		<0.0001***	<0.0001***
Lilleå	0.015	0.021	0.015	0.014	0.016	0.042		<0.0001***
Lindenborg	0.006	0.016	0.012	0.012	0.012	0.032	0.016	

Discussion

The movement of individuals between populations can have varying levels of impact to population demography and recruitment, and consequently population sustainability (Bett et al., 2017; Bowler & Benton, 2005). In this study, an interdisciplinary approach of combining otolith microchemistry with genetics was used to estimate the degree of straying and gene flow among sea trout originating from four rivers flowing into the same fjord system. Otolith microchemistry gives the opportunity to determine the patterns of connectivity over the course of an individual's life, while genetics can document these patterns over evolutionary time.

To determine the origin of adult mature sea trout captured in freshwater during spawning season, river specific baseline otolith microchemistry fingerprints were determined for each river in Mariager fjord using juvenile brown trout otoliths. Juvenile brown trout otolith elemental ratios were used to build a Random Forest classifier, which was subsequently used to assign spawning adult sea trout to their river of origin. The classification accuracy of the Random Forest classifier, which included $^{87}\text{Sr}/^{86}\text{Sr}$, Sr/Ca, Ba/Ca, Mg/Ca and Mn/Ca, was 80%, which is a high accuracy for classifying individuals to their river of origin, resembling results from previous studies. For example, in otolith microchemistry studies among Pacific salmonids, a classification accuracy between 89 and 100% (Maguffee et al., 2019; Turcotte & Shrimpton, 2020) has been documented, while other studies on brown trout have reported accuracies between 73 and 93% (Matetski et al., 2022; Mikheev et al., 2021). Our study therefore provides further evidence that otolith microchemistry can be used to differentiate juvenile trout originating from different rivers. Remarkably, the differences in otolith microchemistry between juvenile trout originating from different rivers in Mariager fjord were detectable over small spatial scales, as the distance between the mouths of the rivers in this system ranged between 4 and 16 kilometres.

The classification accuracy for assigning juvenile trout to their natal river was not uniform across rivers, as it varied between 66 and 95%. Individuals originating from the rivers situated on northern side of the fjord, Villestrup and Valsgaard, had higher likelihood of being correctly classified to their natal river than individuals from rivers on the southern side, Kastbjerg and Maren Møllebæk. While overall high classification accuracy has been reported for different systems (Mikheev et al., 2021; Turcotte & Shrimpton, 2020), it has also been documented to be accompanied with high variability in classification accuracy for nearby rivers (Matetski et al., 2022). Surprisingly, individuals from the river Kastbjerg, which had the lowest classification accuracy, were most often misclassified to originate from river Villestrup, which is located on the opposite side of the fjord, indicating that geographical proximity is not the only factor affecting classification accuracy. While local bedrock is considered an important factor affecting elemental composition of otoliths (Campana, 1999; Goldstein & Jacobsen, 1987), especially concerning $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr/Ca (Barnett-Johnson et al., 2010; Brown & Severin, 2009), other factors, for example presence of glaciated sediments (Frei & Frei, 2011) and fertilizers (Zieliński et al., 2016), which can affect $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr/Ca values, may also have an effect in this system.

No single element had the ability to discriminate among all the rivers, supporting the importance of a multi-elemental approach. Among all the elements included in the classifier, $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr/Ca contributed the most to classification accuracy, while Ba/Ca , Mn/Ca and Mg/Ca contributed less. The importance of $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr/Ca has been documented in similar studies among different taxa originating from various systems (Heidemann et al., 2012; Matetski et al., 2022; Mikheev et al., 2021). During the analytical process, additional elements were measured from the otoliths (Pb, and Zn), but did not improve, and in some instances negatively affected, the ability of the Random Forest classifier to assign individuals to their natal river, so they were excluded from the analysis.

Assigning spawning adult sea trout to their natal rivers

Otolith microchemistry analysis estimated that $43\% \pm 2$ of the adult sea trout that had returned to freshwater originated from another river than the one they were collected in, meaning they had strayed. While, there is a level of uncertainty associated with the estimated proportion of strayers due to possible misclassification of individuals, these results coincide with the findings from previous studies in this system, which documented straying rates between 12 and 55% using PIT telemetry (Källo et al., 2022a, 2023a). All the strayers in this study were collected in freshwater during the spawning season and were determined to be mature, indicating that these individuals had likely returned to the given river with the aim to spawn. However, some of them may have also returned to their natal river, or another non-natal river, prior to being captured in the non-natal river, as has previously been documented (Källo et al., 2022a, 2023a). Unfortunately, such fine-scale movements using otolith microchemistry were not investigated in this study, as only the section corresponding to the juvenile stage in freshwater were analysed. It is however unclear whether such patterns would be detectable if the entire cross section of the otolith would be analysed, as extended time in freshwater is required for the elements to be embedded in the otolith.

The proportion of strayers was high in all of the rivers (between 36 and 55%), with no differences in the proportion of strayers and natal homers among the rivers. This indicates that strayers make up a significant proportion of the spawning contingent in all of the rivers in Mariager fjord. There was also some indication that straying was more prominent towards the larger rivers Villestrup and Kastbjerg, which has also been documented previously in this system (Källo et al., 2023a). While similar patterns of straying towards larger rivers have also been reported in other systems (Degerman et al., 2012; Unwin & Quinn, 1993), straying seems to occur predominantly between rivers in close proximity (Berg & Berg, 1987; Jonsson et al., 2003). However, as all the rivers in Mariager fjord are within the distance over which straying

commonly occurs (60 – 80 kilometres; Bekkevold et al., 2020; Jonsson et al., 2003), it may indicate that in close proximity to the natal river, other factors, such as river size and individual characteristics (e.g. length), affect patterns of straying.

The likelihood of straying depended on the length of adult sea trout, but patterns differed between rivers of origin. Longer individuals originating from Valsgaard and Kastbjerg were more likely to stray, while shorter individuals were more likely to stray if they originated from Villestrup. Nothing can be said about individuals originating from Maren Møllebæk due to a low number of strayers originating from the river being captured. While it is unclear which factors affect the documented patterns of straying, several hypotheses can be formulated, with the causes possibly differing among rivers. For example, in river Villestrup, where the spawning population is large (Birnie-Gauvin et al., 2018; Källo et al., 2023b) and competition for mates and adequate spawning habitat is likely high, it may be more beneficial for smaller fish originating from Villestrup to stray to non-natal rivers, where they may have a better chance of spawning successfully. In contrast, for individuals originating from river Valsgaard, the decreased likelihood for larger individuals to return to their natal river may suggest that the size of the river limits the inclination for larger sea trout to return. River Valsgaard is a shallow river with relatively low flow (Källo et al., 2023a), which may make it difficult for larger sea trout to enter it. However, this hypothesis likely does not apply to individuals originating from river Kastbjerg, where similar pattern of larger individuals straying to a higher degree was documented, given that in size, it is more similar to river Villestrup than Valsgaard. Thus, it is unlikely that the size of the river is the factor affecting the documented pattern, with further research required to determine the true mechanisms behind these patterns.

We further investigated whether straying in this system was sex-biased, as has been previously documented in salmonids (Hamann & Kennedy, 2012; Turcotte & Shrimpton, 2020). While there was some evidence for the sex of the individual to have an effect on straying likelihood,

possible bias stemming from misclassification and wide confidence intervals leave little to no evidence to support the notion that straying in this system is sex-biased. This is somewhat surprising as male salmonids have often been documented to stray more (Hamann & Kennedy, 2012; Turcotte & Shrimpton, 2020), with differences in reproductive strategies suggested as a driver for this pattern (Hard & Heard, 1999). However, Unwin and Quinn (1993) have also documented no effect of sex on straying among hatchery Chinook salmon (*Onchorhynchus tshawytscha*) released to river Rakaia, New Zealand, perhaps indicating that the effect of sex differs between populations or species of salmonids. However, despite proportionally similar straying rates between sexes, it is likely that *per capita* there are more female strayers, as anadromy is more prevalent among female sea trout (Klemetsen et al., 2003; Ferguson et al., 2019). Having more female strayers would imply greater impacts of straying on population dynamics and recruitment in this system.

Genetic structure of sea trout in Mariager fjord

The results of the genetic analysis indicate that individuals originating from different rivers in Mariager fjord likely make up a single breeding population and that this is consistent over generations. More specifically, it was documented that there was a stable gene flow among rivers within Mariager fjord, which indicates that strayers migrating to non-natal rivers are able to successfully spawn there. However, the reproductive success of strayers cannot be determined with the applied genetic method, as straying of only a low number of individuals per generation will eradicate genetic signals of demographic structure (Waples & Gaggiotti, 2006).

In contrast, gene flow (successful reproductive straying) in Mariager fjord seems to be mainly confined to the rivers within the fjord, as shown by the clear genetic differentiation between collections from Mariager Fjord and those from the neighbouring rivers (river entrances

separated by ca. 10-45 km). This finding supports the notion that (reproductively successful) straying is generally more prominent within local areas, and decreases in prevalence the further apart rivers are located. However, as mentioned above, the rivers investigated in this study (both inside and outside of the fjord) are within the distance over which straying has previously been documented (Bekkevold et al., 2020; Jonsson et al., 2003), perhaps indicating that the fjord acts as a barrier between the two areas, limiting gene flow. The latter may be partly also supported by findings of del Villar-Guerra et al. (2014), who documented that significant proportion of sea trout never leave Mariager fjord.

Conclusion

In conclusion, otolith microchemistry combined with genetics provides an important tool for investigating population connectivity across the life of individuals and over evolutionary time. In this system both methods indicated there was a substantial exchange of individuals and genetic material between the rivers. Further, this study contributes to the growing body of evidence that otolith microchemistry can differentiate between populations originating from different rivers, even at relatively small spatial scales. By comparing adult sea trout otolith fingerprints to juvenile river-specific otolith fingerprints, we were able to assign adult sea trout to their river of origin, and additionally confirms that strayers make up a significant proportion of the spawning population in all the rivers within the Mariager fjord system. We further identified individual length and river of origin as important factors affecting straying. A lack of genetic structuring between the rivers in Mariager fjord confirms that the strayers in this system are able to successfully spawn in non-natal rivers, and that all the rivers make up a single population rather than separate, genetically distinct populations.

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Author contribution

KK and KA contributed to the conception and the design of the study. KK carried out the fieldwork. KK, PG, CL and GB carried out the laboratory analysis. KK, HB and DB analysed the data. All authors contributed to the interpretations of the results and have given their final approval for the manuscript to be published.

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Data Availability:

Data will be available from the authors upon reasonable request

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Supplementary material: "Otolith microchemistry combined with genetics reveal patterns of straying and population connectivity in anadromous brown trout (*Salmo trutta*)"

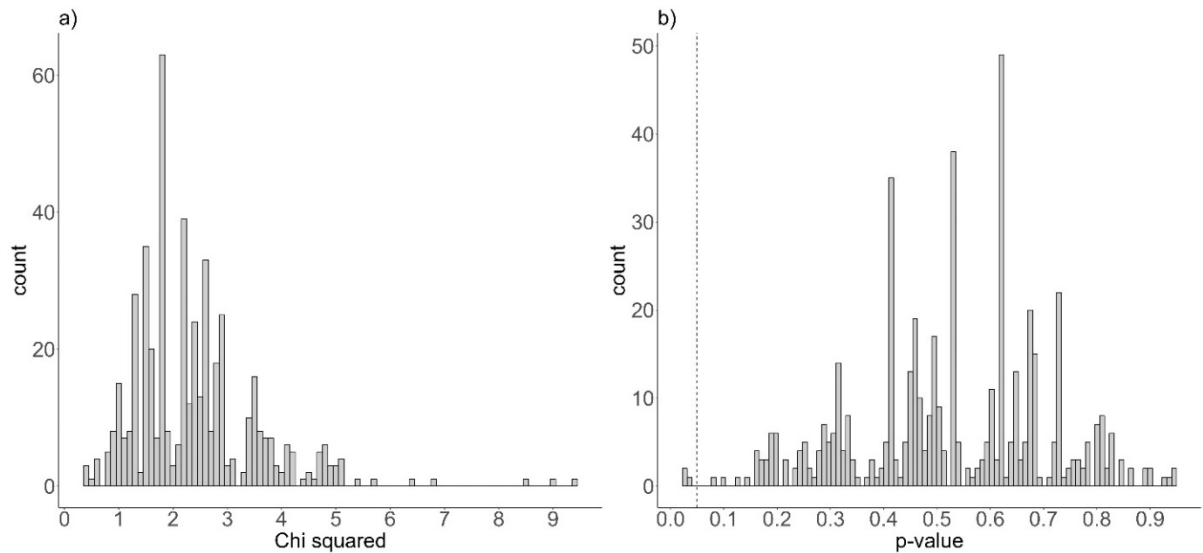


Figure S1. Histogram of the a) chi squared and b) p-values from Chi-squared test (df=3) for each of the assignment datasets, attesting to differences between the proportion of natal homers and strayers in each of the rivers. The dashed line on plot b) represents $p=0.05$.

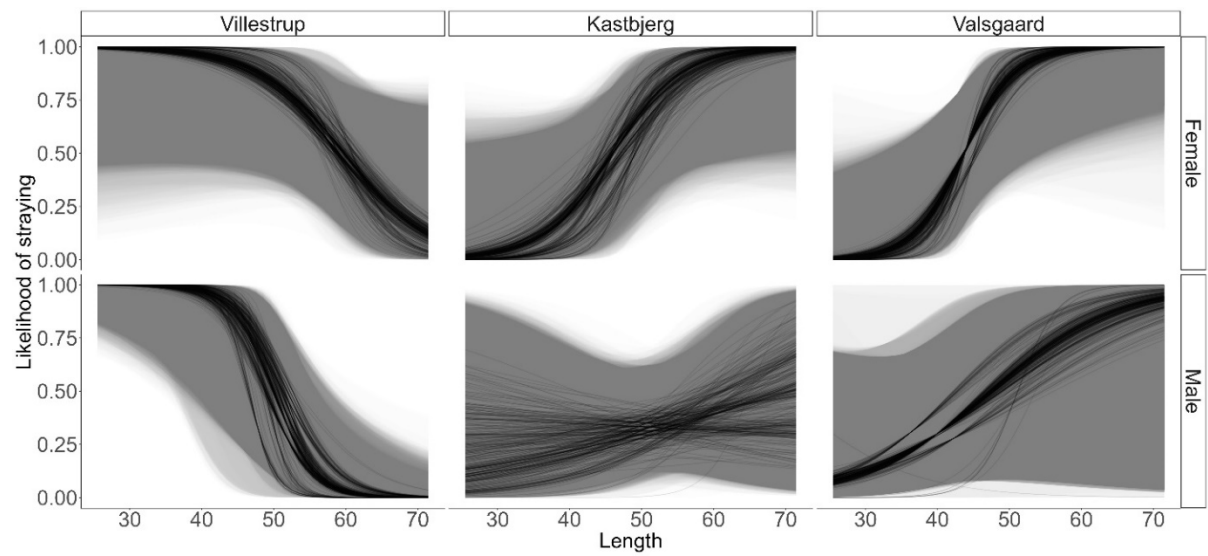


Figure S2. Modelled output of the likelihood of straying for mature sea trout against length at capture, divided by river of origin and sex. The lines and the shaded area represent the mean and 95% confidence intervals for each of the models.

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