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Behaviour and saltwater tolerance of European whitefish *Coregonus lavaretus* (L.) in an Arctic estuary

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Acknowledgments

I want to thank all people who passionately fight to understand and protect our rivers, our lakes and our seas, to keep them as pristine as they should be, against the alienation of some, inspired by other values, underestimating this endless source of joy, food and inspiration.

Chief Seattle's letter to the president of the United States, 1852

« *The President in Washington sends word that he wishes to buy our land. But how can you buy or sell the sky? The land? The idea is strange to us. If we do not own the freshness of the air and the sparkle of the water, how can you sell them? Every part of this earth is sacred to my people. Every shining pine needle, every humming insect. All are holy in the memory and experience of my people. »*

(...)

« The shining water that moves in the streams and rivers is not just water, but the blood of our ancestors. If we sell you our land, you must remember that it is sacred. Each ghostly reflection in the clear waters of the lakes tells of events and memories in the life of my people. The waters murmur in the voice of my father's father. The rivers are our brothers. They quench our thirst. They carry our canoes and feed our children. So you must give to the river the kindness you would give any brother. »

(...)

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Thank you

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Abstract

European whitefish is well described and renowned for its polymorphism and faculty to occupy a wide range of freshwater habitats. This includes observations of individuals in some populations that utilize brackish estuarine habitats as feeding areas, although information on these populations is generally scarce. By use of acoustic tracking technology and physiological sampling, the behaviour and salt water tolerance of European whitefish was therefore studied in the estuary and the adjacent fjords of the River Neiden in Northern Norway where individuals have earlier been captured in the estuary area. The results showed that European whitefish utilized the estuary extensively as a feeding habitat, with the capacity to stay for short periods in salinity concentration up to 30 ppt and with a mean water temperature of 15 ˚C. A correlation was found between their horizontal distribution along the estuary axis and the salt water influx following the tidal variations; fish were mainly using the lower estuary at lower salinity concentration following the tidal level, and retreat upstream with the salt water influx under high tide. This study therefore demonstrated that European whitefish at it northernmost distribution area can utilize the estuary extensively as a feeding area. There was also indications of that at least a few individuals could possibly cope with relatively high salinity concentration over some time. This is the first study that has mapped the individual behaviour of European whitefish in an estuarine area over time and therefore provides a better understanding on the ability of the species to cope with salt water habitat. Given that these populations are genetically unique, they should be given special management emphasis.

Introduction

The European whitefish (*Coregonus lavaretus* (L.)) is mainly present in cold and clear freshwater. It has its natural range in the Eurasian Arctic and Sub-Arctic, as well as some populations are found in the Alpine areas of Europe and on the British isles (Pethon 2005). It is known for its considerable degree of polymorphism and its ability to adapt to different ecological niches in lakes or in rivers (Østbye et al. 2006, Huuskonen et al. 2012). Although European whitefish regularly is thought to be restricted to freshwater habitats, several anadromous[1] populations are described: in the Baltic sea, on the west coast of Denmark and in northern Norway (Lehtonen, 1992, Czerniejewski & Rybczyk 2009, Poulsen et al. 2007, Staldvik 1989). As far back as in 1918 it was suggested that European whitefish colonized south-western Norway using the coastal brackish water at the end of the last ice age (Huitfeldt-Kaas, H. 1918). Fishery of European whitefish was the third most important in the brackish water of the Limfjord on the west coast of Denmark, before an increase of the salinity to above the whitefish physiological tolerance level due to the break of a narrow isthmus at the outlet of the fjord (Poulsen et al. 2007). European whitefish is considered as one of the most important species for the coastal fisheries in the Gulf of Bothnia, the northeast part of the Baltic Sea, where two different populations are found, a population of river-spawner and one of sea-spawner (Lehtonen & Himberg 1992).

Under laboratory conditions, by exposing river-migrating European whitefish to different degrees of salinity, Madsen et al. (1996) demonstrated that European whitefish acclimated successfully to 25 ppt, but died after direct transfer to 32 ppt sea water. In this experiment, European whitefish that were exposed to salt water, showed physiological responses reflecting the initiation of hypo-osmoregulatory mechanisms; such as an increase of Na^+,K^+-ATPase activity in the gill or an increase in plasma cortisol concentration. It is important to underline that these mechanisms are the result of an exposition to salt water and not the one of a pre-adaptation such as a parr-smolt transformation seen in other anadromous salmonids (Hoar 1976), which has not been documented for *Coregonus lavaretus* (Madsen et al. 1996). As European whitefish larvae have been shown to drift downstream from their hatching site in the river to the sea (Lehtonen et al. 1992), the physiological response to salt water exposure may occur in the very early stages of development. Even though this drift is influenced by an increase of the river discharge, it does not seem to be completely passive, and to vary between different environments (Lehtonen et al. 1992, Næsje et al. 1986). This suggests that not all larvae automatically are flushed into salt water and it may depend on the river configuration and the strength of the spring flow. In a proteomic study, Papakostas et al. (2012) compared individuals from two different populations of European whitefish: a sea-spawning population from the Baltic Sea and a population restrained to freshwater habitat from an inland lake. They showed that, even if the two populations had a low level of genetic differences, their phenotypic and proteomic responses to salt water exposure were highly different. During the fertilization and embryonic phases, survival of the freshwater population in salinity of 6 and 10 ppt was 40 % lower than the survival of the saltwater's population. This indicates population variability among European whitefish from different environments in their ability to tolerate salt water.

Several salmonid species have been recognized to differ in their range of temperature optimum, and to be directly affected by temperature for their habitat choice at sea (Azuma 1995, Jensen et al. 2014). Although, low temperatures in the marine environment are known to affect negatively the physiological response to salt water or the growth rate of several salmonids species (Finstad et al. 1988, Handeland et al. 1998), little is known about the correlation between the water temperature and the salt water tolerance of European whitefish. Madsen et al. (1996), Papakostas and co-workers (2012), used a constant water temperature of 14 ˚C and 6 ˚C to demonstrate the salt water tolerance of adults and embryos of European whitefish in their experiments.

Salmonids such as brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus* show an increasing degree of anadromous behaviour towards higher latitudes (McDowall 1997, Klemetsen et al. 2003). The sea is generally a more productive habitat than freshwater systems at these latitudes, and allows migratory fish to increase their fitness and thereby their reproduction rate (Gross et al. 1988). In estuaries, detailed studies have shown an increase in both biomass and diversity fallowing a salinity gradient from fresh to salt water (Ysebaert et al. 1993, Josefson & Hansen 2004). As the freshwater runoff generally decreases in the estuary during summer due to a lower mean flow in the river, the salinity increases in the estuary. Following this, the brackish-water and marine species gradually increase in abundance inside the estuary during summer and fall (Attrill & Rundle 2002). Furthermore, crustacean (amphipods) that have been found to play a major role in European whitefish diet in the brackish environment (Staldvik 1989, Verliin et al. 2011), can show a peak of abundance in the brackish part of several Nordic estuaries (Chertoprud et al. 2004, Ysebart et al. 1993). Migratory fish entering saltwater have to find the right balance between the gains and the costs: such as energy and time lost, osmotic costs, and increased predation risk (Helfman et al. 2009). In estuaries, one of the main challenges could be to physiologically cope with the continuous change in salinity, which requires an ability of the fish to alter from hypo- to hyper-osmoregulation, and the related energy cost (Whitfield 2015).

Staldvik (1989) documented a population of anadromous European whitefish as far north as in the River Neiden in Norway (69 °N). In this study, European whitefish were caught by gill-nets at different stations in the estuary and in the adjacent fjord. Anadromy in European whitefish is particularly well described in the Gulf of Bothnia (e.g. Lehtonen & Himberg 1992, Leskelä 2006), where the salinity varies between 3 and 7 ppt (Håkansson et al. 1996). Less is known about populations having a more direct access to marine areas with a higher level of salinity and a restrained brackish water stretch (Jonsson et al. 1988). The River Neiden and the adjacent fjord system are directly connected to the high salinity water of the Barents Sea. In such a system, the migratory salmonids are exposed to an elevated level of salinity after a short displacement. In contrast to the laboratory experiments, such as the one conducted by Madsen et al. (1996) where fish were seen to develop physiological response following a relatively long salt-water exposure, this natural environment allows the fish to freely move between salt- and fresh water. Investigating their physiological status and behaviour within this environment, allows to examine in which extent European whitefish use the more salty part of the estuary and fjord or retreat to fresh water to avoid physiological stress (e.g. an increase of salts concentration in the blood plasma).

In order to map with accuracy the habitat use and the behaviour of fish individuals, continuously and over long time, electronic telemetry has shown a great advantage over other methods (Thorstad et al. 2013). To my knowledge only two studies have been done using this technique in order to examine European whitefish behaviour and habitat use; one in a small Belgian artificial lake (Ovidio et al. 2006) and the other in a Finish river (Hannu et al. 2012). No study of this kind have been done on European whitefish in the brackish environment, and very little is known about their behaviour and habitat use in a constantly changing habitat with regular strength salt water influx.

Based on the background described, the main aims of this study are to analyse the behaviour and the salt water tolerance of European whitefish in a complex environment; the estuary and the adjacent fjords of the River Neiden. The sub goals are, firstly, map their horizontal, vertical and temperature use in the different parts of the estuary and in the fjord, secondly; compare their habitat choice with regard to the tidal/salinity variations, and finally, examine their gill ATPase activity level in order to relate their habitat choice to the tidal and salinity variations. In addition a salt water challenge was done under controlled conditions, to test their tolerance to a direct transfer in natural sea water.

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1. Referring to Lehtonen & Himberg (1992), use the term “anadromous” to a larger extent by considering migration to brackish water as a form of anadromy.

Material and method

Area description

The River Neiden has its source in the Lake Lijärvi in northern Finland and connects the sea in eastern Finnmark Country in northern Norway (69° 70' N, 29° 58' E). It has a catchment area of 2980 km² with an annual mean flow calculated for 2014 of 34.3 m³/s (min: 9; max: 214) (source: Norwegian watercourses and energy directorate). Based on Arnessen (1987) and own observations, the river fish fauna consists of 13 native fish species; brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), European whitefish, Arctic grayling (*Thymallus arcticus*), river lamprey (*Lampetra fluviatilis*), pike (*Esox lucius*), burbot (*Lota lota*), European minnow (*Phoxinus phoxinus*), three-spined stickleback (*Gasterosteus aculeatus*), nine-spined stickleback (*Pungitius pungitius*), European flounder (*Platichthys flesus*). Two non-native species are also regularly observed within this system: rainbow trout (*Oncorhynchus mykiss*) escaped from fish farms and pink salmon (*Oncohychus gorbusha*) introduced on Kola Peninsula (Russia). A large waterfall, Skoltefossen, is located approximately 12 km upstream from the river outlet, characterized by 3 steps of *c.* 1-2 m fall each, with strong currents and pools in between. This waterfall does not stop upstream movement of Atlantic salmon or sea trout, and migration is enhanced by a fish ladder located on the north side of the last step. Whether the waterfall constitutes an upstream barrier for European whitefish remains unknown, but in a report on annual video monitoring on the waterfall's effect on fish movement done between 2006 and 2011 from mid-June to August there is no mention of any upstream movement of European whitefish (Orell 2012).

The River Neiden estuary ranges from 300 – 600 m in width, and the water depth in the main channel ranges from 1 to 5 m in some deep pools at low tide. A large accumulation of sand at the outer side of the delta almost obstructs the Neidenfjord leaving a short strait of *c*. 200 m in width and 13 m in depth connecting Neidenfjord with Munkefjord (Fig.1). In addition, a small channel splitting from the main channel creates another connection between the lower part of the estuary and Munkefjord (Fig.1). The lower part of the estuary is characterized by strong upstream or downstream currents during the intertidal period, with slower currents at high or low tide. The tidal currents decrease upward but can still be observed *c*. 8 km upstream. Due to the difference in water densities, freshwater income from the river is pushed *c*. 8 km upstream by salt water at high tide, but the limit of the salt water penetration is at *c.* 6 km upstream from the sea at normal high tide (personal observation).

Munkefjord is approximately 1 km wide and has a maximum depth of *c.* 23 m in front of the River Neiden estuary outlet and an average depth of *c.*10 m. Munkelva is another river of importance for migratory salmonids in the study area, and has its outlet in the inner part of Munkefjord (Fig.1). Neidenfjord has a depth of 35 m just outside the estuary, increasing to *c.* 60 m at its deepest and is *c.* 2 km wide.

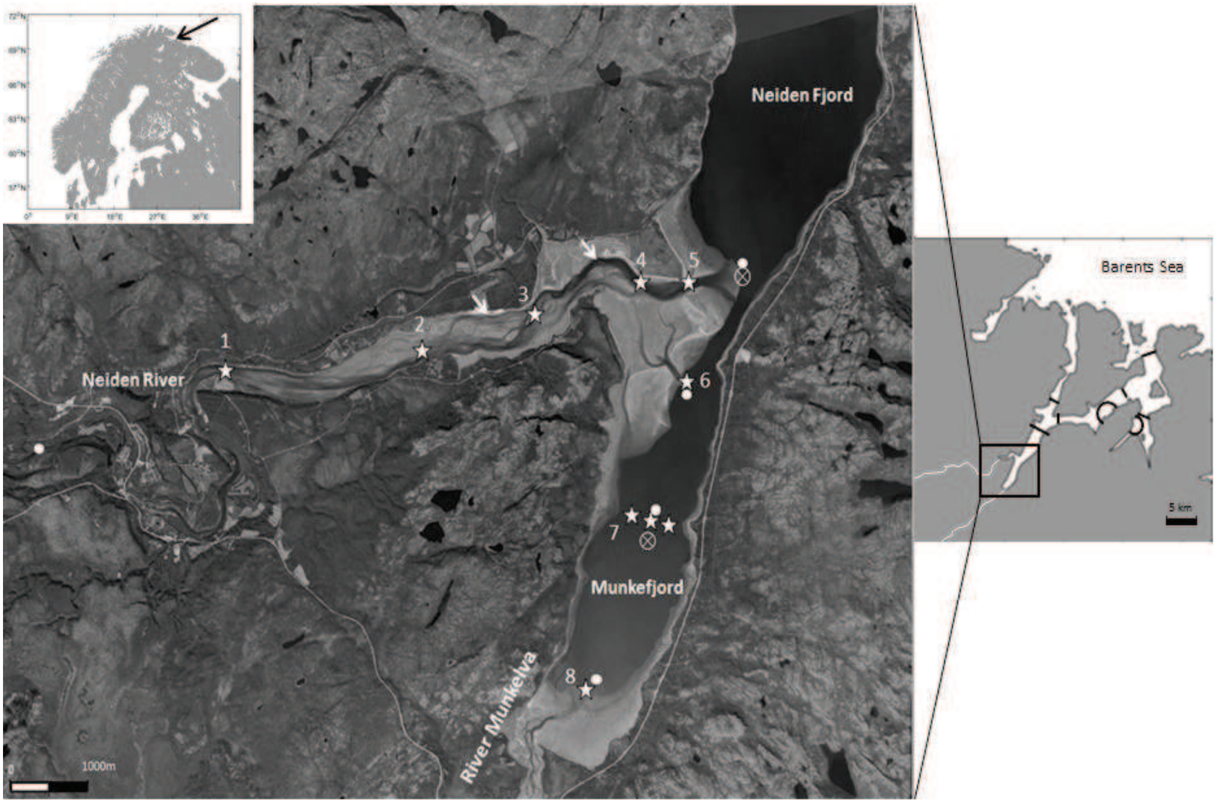

FIG. 1 Aerial photo of the study area at low tide, that shows the sites of capture (arrows), the receivers positions (stars), the sites of temperature (white dot) and conductivity (circle with cross) recording. The release sites are similar to the capture sites (arrows), the lower one corresponds to the position of the cage where fish were kept until tagging. The black lines in the smaller map to the right correspond to transects with receivers used in another project.

Environmental recordings

Salinity in the estuary and fjord

A conductivity-temperature-depth measurement was done on 4 July, by using a hand-held YSI 85CE thermosalinometer (YSI Inc., Yellow Springs, OH, USA), under low wind and wave conditions, which covered the entire estuary (*c*.10 km from the outlet). It showed both vertical and horizontal salinity variations; the surface was mainly dominated by fresh water in the upper estuary with a vertical increase in salinity in deeper water. The variations in salinity showed a more horizontal progression further downstream due to more mixing of the water layers. The salinity values given in Fig. 2 are relatively low, because the measurements started 1:45 hr and finished 4:00 hrs from high tide at decreasing tide and were conducted under a spring river flow of 32.6 m³/s (Fig. 2). At high tide under lower river flow, the same pattern of horizontal salinity progressions might be seen further upstream and with more strength.

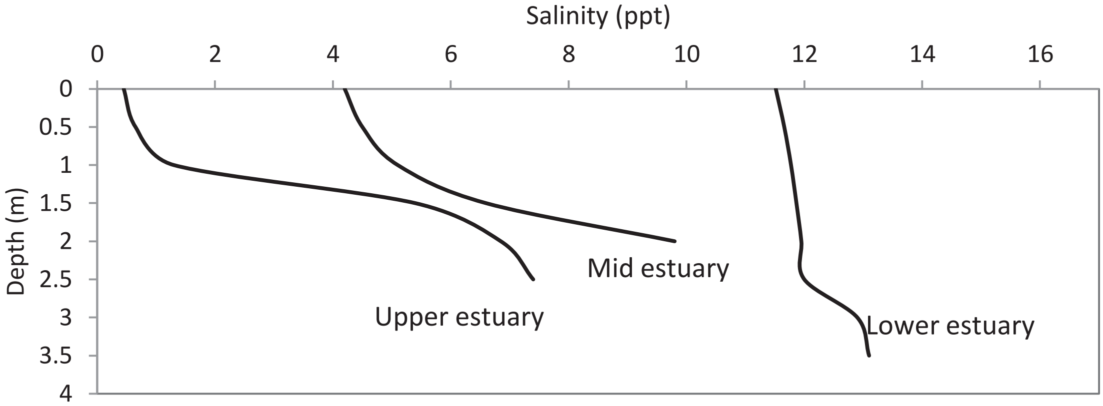

FIG. 2 Horizontal and vertical variations of the salinity along the estuary from upstream to downstream. The salinity variations are given by the mean of the measurements in each zone of the estuary.

Salinity was continuously recorded by two fixed stations every 30 min at 2 m depth, using two conductivity data loggers (Oneset Computer Corporation, Massachusetts, USA; model Hobo U24-002-C); one located at the estuary outlet and the other in Munkefjord (Fig.1). The salinity measured in the lower estuary was clearly impacted by both tidal variations (Fig.4) and the river discharge, even if measured at 2 m depth (Fig.3). The mean salinity at the lower estuary was relatively low (mean; 12.2 ppt, S.D. = 8.6) during the spring river flood (mean flow 73 m³/s, 1 June – 15 July), but increased during summer to an average of 30.6 ppt (S.D. = 3.8) from 15 July to 5 October under a mean river flow of 19.5 m³/s. Even if the salinity was recorded 500 m downstream to the most downstream acoustic receiver, the conductivity measurement at the receiver position at 2 m showed very little differences between the two locations. Mean salinity measured at 2 m depth in mid-Munkefjord was lower both during spring and summer than at the outlet of the estuary and was much lesser impacted by the tidal variations (Fig.3).

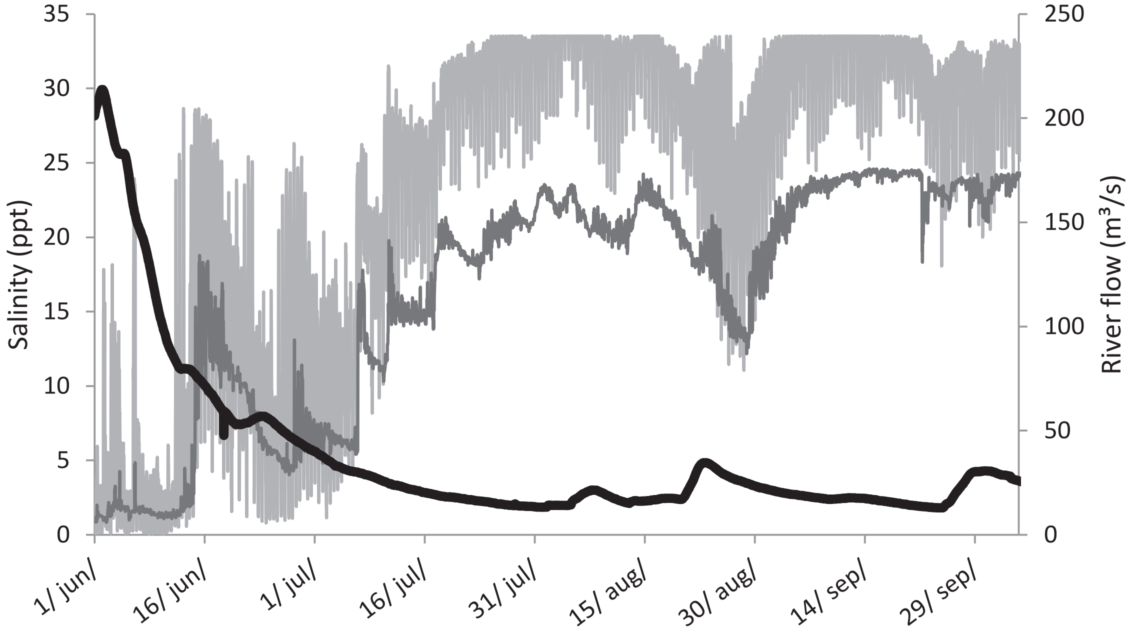

FIG. 3 Relationship between the river flow (black line) and the salinity recorded at the lower estuary (light grey) and in Munkefjord (dark grey).

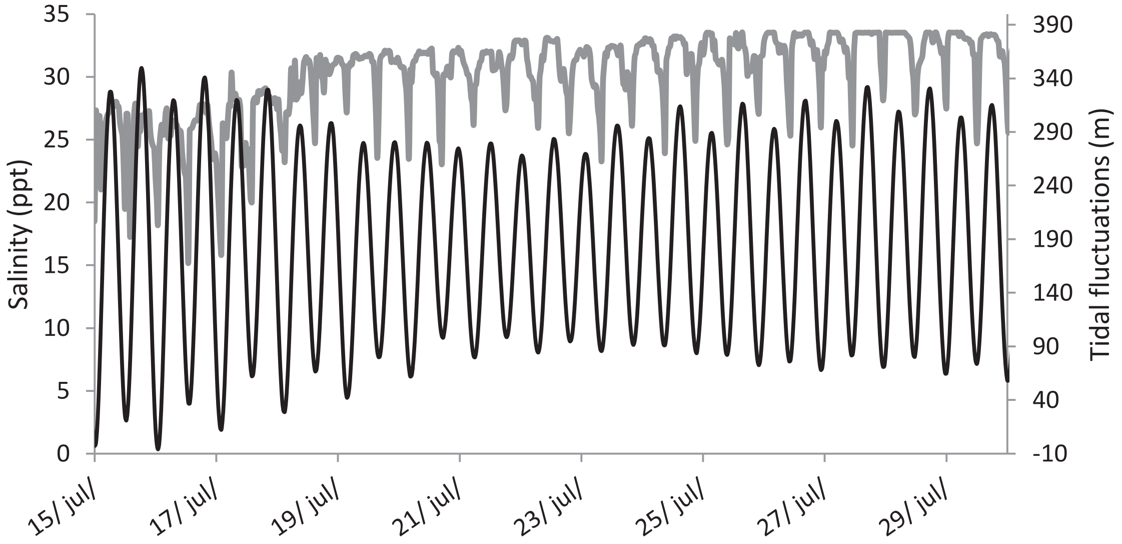

FIG. 4 Relationship between tidal variations and the salinity recorded at 2 m deep in the lower estuary from late spring flood to the summer patterns (15- 29 July).

Temperatures

In the estuary and fjord, temperature was measured every 15 min by the recorders Hobo 64k-UA-002-64 (Onset Comp. corp.) at four locations; the estuary outlet, the outlet of the channel connecting Munkefjord, in the middle of the acoustic receiver transects in Munkfjord and in the inner part of Munkefjord (Fig.1). At each location, one temperature recorder was placed at each of the depths; 1, 3, and 5 m from the surface fixed vertically on a rope below buoys and anchored to the bottom (loggers located at 5 m depth at the outlet and mid-Munkefjord lost their data). In the river, temperature and water flow were recorded each hour by the Norwegian watercourses and energy directorate at a station located *c.* 10 km upstream from the estuary outlet (Fig.1).

Temperature during the spring flood (1 June – 10 July) was lower in the river (9.9 ˚C) than in the upper 3 m of Munkefjord and lower estuary (mean between 1-3 m; 10.5 ˚C). From mid-July until the end of August the river temperature was higher than the estuary's outlets and Munkefjord at all depths (mean river; 15.5 ˚C, estuary and fjord 1 m; 12.4 ˚C, 3 m; 11.5 ˚C, 5 m; 9.7 ˚C). The temperature decreased by the end of August to October (mean river; 8.6 ˚C, estuary and fjord 1 m; 9.1 ˚C, 3m; 9.0 ˚C, 5 m 8.8 ˚C). Temperature measured at 5 m depth in the lower estuary and Munkefjord was always lower than in the river (mean: 8.3 ˚C) expected from late august where it was firmly similar to the river temperature.

Behavioural study

Tagging and recording

In order to map the horizontal and vertical movement and the temperature experienced by European whitefish, a total of 22 individuals (mean fork length (L_F) 396 mm, range 320 - 470, mean mass 898 g, range 530 - 1300) were captured and tagged in the estuary between the 16 June and the 3 July 2014 (Fig.1 for capture locations). Twenty-one of the tagged fish were captured by monofilament gill nets (35 to 52 mm knot to knot) and one by rod. While fishing with gill nets, the nets were regularly inspected (*c*. every 10 minutes) in order to avoid injuries to the fish. The tagged fish had less than 4% scale loss on average and those with more than 8% scale loss were not tagged. Fish were either kept up to a maximum of 3 days in a cage located approximately 1 km downstream of the catch location (see Fig.1) until tagging (n=13), or tagged immediately after capture at the catch location (n=9).

The fish were equipped with V13TP-1L acoustic transmitters (cylinder shaped, 13 x 48 mm, 13 g in air, signal interval of 30/90 s, estimated battery life of 1339 days (produced by Vemco Inc., Canada). The tags transmitted signals with sensor information on the depth (i.e. pressure) and temperature experienced by individual fish when recorded when they were within range of the listening stations. All tags where surgically implanted in the body cavity through a small incision in the abdomen behind the pelvic girdle. The incision was closed by 2-3 independent silks sutures (3-0 Ethicon). Prior to the surgical implantation, all fish were anaesthetized in 2-phenoxy-ethanol (SIGMA Chemical Co., USA; EC No 204-589-7, 0.3 ml/l, mean time in anaesthesia 3 min). During tagging fish were kept with head and gills in water in a half tube with the dorsal side down. Before release, fish were weighted and length measured. A piece of gill filament (~ 1 mm) was taken for ATPase analyses (McCormick 1993) and a piece of the adipose fin was collected for genetic analyses (<4 mm). The genetic samples were kept for further studies but were not taken into account in this study. All tools used for the surgical operation were disinfected in 96% ethanol. The total handling and tagging time per fish, including anaesthesia, lasted 5-8 min. After tagging and sampling, the fish were kept in a cage in the river and monitored until they recovered and were able to swim by their own. This usually took 5-10 min.

The transmitters that were implanted in the fish sent two individually coded acoustic signals each 30/90 seconds in the water volume; one of the signals informed on the fish's depth and the other on the surrounding water temperature. As the transmitters were located in the body cavity of each fish, the surrounding temperature was buffered by the body cavity, and the transmitted temperature refers to the average water temperature the fish has been swimming within (see e.g. Reddin et al. 2011). The acoustic signals were detected by automatic receivers (type: VR2-W, Vemco Inc.) placed at strategic locations within the estuary and fjords (Fig.1). The detection range was measured to be within a range of 200-1000 m from the receiver, depending on environmental factors such as waves, currents or haloclines. Following this, in order to maximise the detection rate, receivers were placed at maximum 400 m apart from each other in the fjord transects, and maximum 200 meters from the river/estuary banks. The acoustic receivers were placed at 5 m below the surface in the fjord and at 1 m depth in the river, fixed on ropes anchored to the bottom and maintained straight by buoys at the surface. The fish were recorded from their time of release (16 June – 3 July) until 6 October 2014.

Manual tracking

Four surveys of manual tracking using a receiver equipped with an omni-directional hydrophone (VR60, Vemco Inc.) were conducted from a boat drifting downstream; on 9 and 10 July from 8 km upstream to the outlet of the estuary, on 5 and 6 from downstream of the Waterfall Skoltefossen to the outlet of the estuary. Manual tracking using a submersed hydrophone allows recording of the acoustic signals transmitted in the water column by the nearby fish and detects their location within a range of 200-1000 m depending on waves, currents or haloclines.

Physiology analyses

Physiological analyses focused on the Na^+, K^+-ATPase activity in the gill, as this enzyme has been identified to play the major role as an osmoregulatory mechanism in most euryhaline teleost (Marshall & Grosell 2006). Recording the ATPase activity in the gill of fish sampled in the estuary of River Neiden gives the opportunity to examine if hypo-osmregulatory mechanisms occur among this population of European whitefish. In addition to the sampled fish, the ATPase activity was also recorded the tagged fish, and compared with their habitat choice during the study period. The osmolality is a quantitative measure of the osmotic concentration of a solution, and it takes in account all dissolved charged particles, such as Na^+, Cl^-, Mg^{++}, SO_4^-, and thereby gives indication on the hydration status of the fish. The plasma osmolality and the gill ATPase activity of fish caught in the estuary were compared to the plasma osmolality of fish transferred to natural salt water of 30 ppt, under controlled conditions.

ATPase activity

To measure the gill Na^+, K^+-ATPase activity, a piece of gill filament (~10 mg) was collected from the second gill arch of 11 fish caught in the estuary between 12 June and 10 July (mean mass 682 g, range 391 - 1030, mean fork length (L_F) 363 mm, range 300 - 420). These fish were caught using monofilament gill nets (35 to 52 mm knot to knot), and either sampled directly (n=7) at capture or either kept in a cage in the lower estuary for a maximum of 42 hrs (n=4). No noticeable differences were found between the ATPase activity of fish sampled directly or the one kept in the cage in the lower estuary (the average salinity in lower estuary was at this time very low ~ 12 ppt). The sampled and sacrificed fish were killed by a blow to the head prior to sampling. The same gill filament was taken from the tagged fish and released fish. Filaments were directly immersed in ice cold SEI buffer (0.3 M sucrose, 0.1 M imidazole, 0.02 Na_2EDTA) and frozen at -20°C until analyses in the laboratory (21.10.2014). Gill ATPase activity was analysed following the method described by McCormick (1993) at 25 °C using a Spectra max plus 384 spectrophotometer / plate reader (Molecular Devices, Llc., California, USA).

Salt water challenge and plasma osmolality

On 28 June, five of the fish (mean mass 550 g, range 354 -810, mean fork length (*LF*) 348 mm, range 305 - 405) sampled in the estuary on the 26-27 June, were transferred to natural sea water (30 ppt and 10 °C) in a 200-l tank constantly aerated. Fish were regularly monitored during the 24 hrs of salt water challenge; and their condition and lethality were recorded. Blood samples were taken from the dorsal aorta of each fish for the osmolality measurement, and a piece of gill filament for the ATPase measurement. Samples were either taken from newly dead fish during the monitoring process or from the survivors after being killed by a blow to the head.

In order to compare the osmolality of these fish with the fish using the estuary, blood samples were taken from the dorsal aorta behind the pelvic fin of 23 fish caught in the estuary between 12 June and 8 July (mean mass 717 g, range 347 – 1116, mean fork length *(LF*) 370 mm, range 300 - 434). The plasma was immediately separated from the blood by centrifugation (6000 ×g, for 10 min) and stored at -20 °C until analyses in the laboratory (25.02.2015). The plasma osmolality was measured using a Fiske One-ten osmometer (solid state cooling system) (Advanced instruments, Inc., Massachusetts, USA) and expressed in mosm/kg H_2O.

Data analyses

When analysing the data provided by automatic acoustic receivers and manual tracking, fish were defined as dead or to have lost its tag, when the fish stopped to give any sign of vertical or horizontal movement over several days. The data provided by the fish estimated as dead or to have lost their tag (n=6) were included until the last record of activity.

Considering the salinity distribution and the position of the receivers, the estuary was divided in three different zones; upper, middle, and lower estuary. Fish recorded by receivers 1 and 2 (Fig.1) were defined as occurring in the upper estuary, receiver 3; middle estuary; receiver 4,5; lower estuary, and receiver 6, 7, 8; Munkefjord.

The time of residency within each zones, was estimated as the time between the first and the last registration within the area. Fish used to send regular records to the receivers when occurring in the defined zone. When missing of detection was observed when a fish was swimming from a receiver to another without being detected by the intermediate receiver; it was decided to split the time of residency within a zone when an absence of detection within that area exceed 6 hrs.

The horizontal distribution of the fish was compared to the tidal level and salinity Since the recorded salinity and tide shown non-normal residual distributions, a Mann-Whitney U test was used, with a continuity correction for multiple comparisons. The same test was used to compare the time of residency in the lower estuary and the salinity gradient using a Bonferroni correction. Data computing was done by using Microsoft Excel 2010 and statistical analyses were done by using the free software R (http://www.r-project.org).

Gill Na^+, K^+-ATPase (EC 3.6.1.3) activity was analysed by a standard micro-assay procedure given by McCormick (1993).

Results

European whitefish behaviour within the estuary and fjord

Movement and residency

In general the results show that the European whitefish in the lower River Neiden utilized the estuary actively. Most fish utilized every part of the estuary from the lower to the upper zones (Fig.5), and 5 fish entered the sea to the Munkefjord during the study period. All individuals performed regular downstream- upstream movements, between upper, mid-, and lower estuary during the entire study period (Fig.5).

The tagged fish spent most of their time in the upper and mid-estuary, on average 49% (S.D. = 22%, max = 66 hrs) and 31% (S.D. = 20%, max = 58 hrs) of the time respectively. While 21% of their time was in the lower estuary (S.D. = 19%, max = 57 hrs), and 5% in Munkefjord (S.D. = 6%, max = 10:46 hrs) (Fig.6).

Among the fish that entered the sea, two moved downstream to Munkefjord and after a short period moved back upstream. Fish #4 was detected at the small channel outlet (receiver 6) for 2 min and thereafter moved upstream. Fish # 20 moved across the fjord to the outlet of the River Munkelva, and spend 8 hrs in the fjord before moving upstream again to the upper estuary *c*. 10 hrs later, where it was registered by manual tracking on 5 October on a putative spawning ground in the river. One fish tagged in the lower Neiden estuary the 24 June (#12) presumably entered the River Munkelva the 1 July, after spending between 10:46 (min estimated time) and 28:46 hrs in the fjord. Fish # 13 was last recorded at receiver 6 after spending 7:06 hrs in the area, #10 died or lost its tag close to receiver 7 after spending actively 5:50 hrs in Munkefjord.

Ten of twenty-two individuals were regularly recorded during the entire study period, and were found on a putative spawning ground by manual tracking on 5 and 6 October from *c*. 600 m to 2200 m downstream from the Waterfall Skoltefossen. These ten fish were the only fish that were last recorded at the most upstream receiver (receiver 1) between 16 July and 12 August (average: 27 July).

Five individuals were last recorded in the lower part of the estuary ($n = 4$) or in Munkefjord ($n = 2$) (receiver 5 or 6) from 1 to 13 days after their release. Six fish lost their tag or died between 1 and 27 days after their release (average: 15 days) indicating a minimum estimated survival of 73 %. No significant difference were found between the condition (Fulson's K factor) of these individual with the ones that were defined as alive, Randomization test ($p > 0.05$).

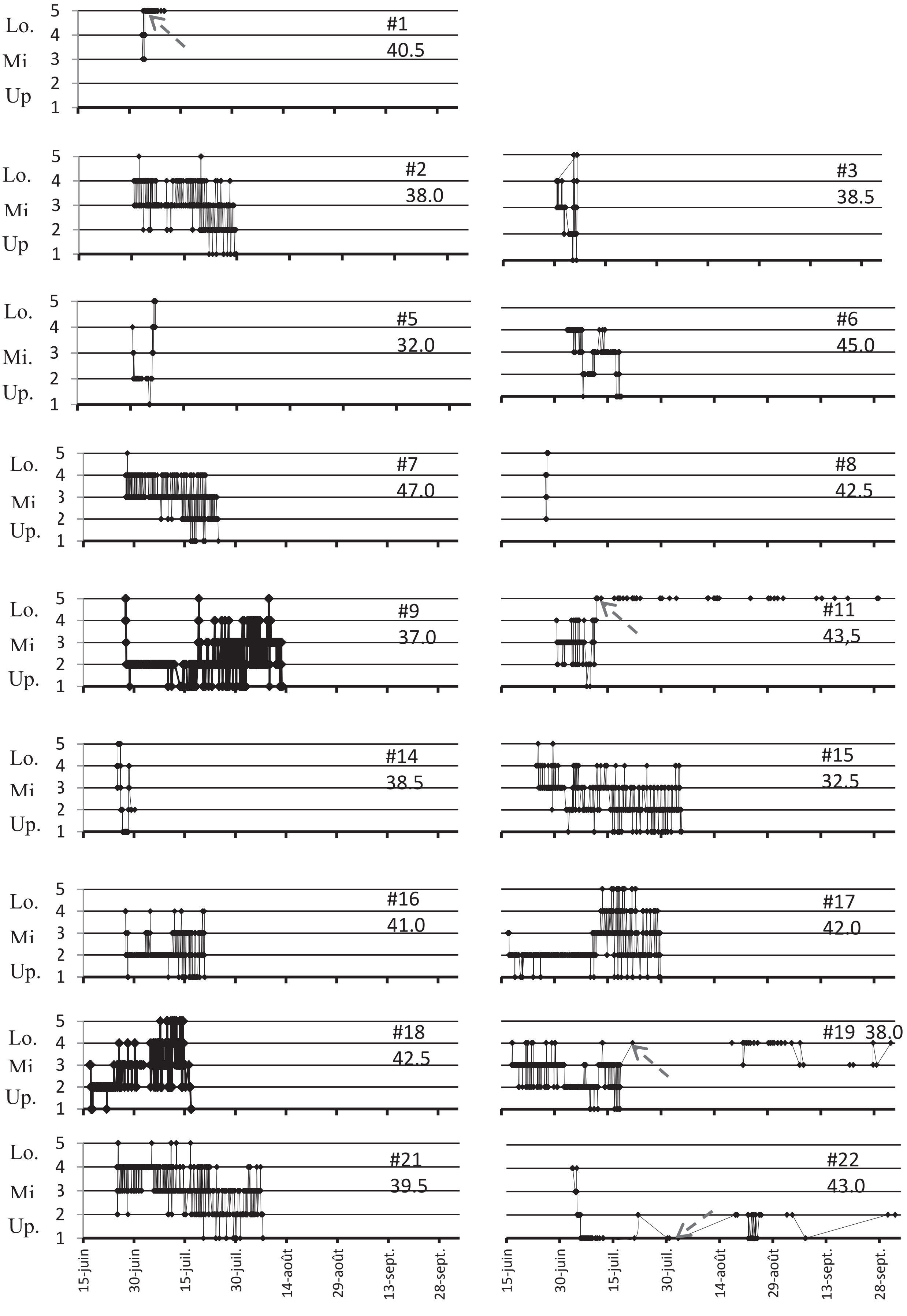
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FIG. 5

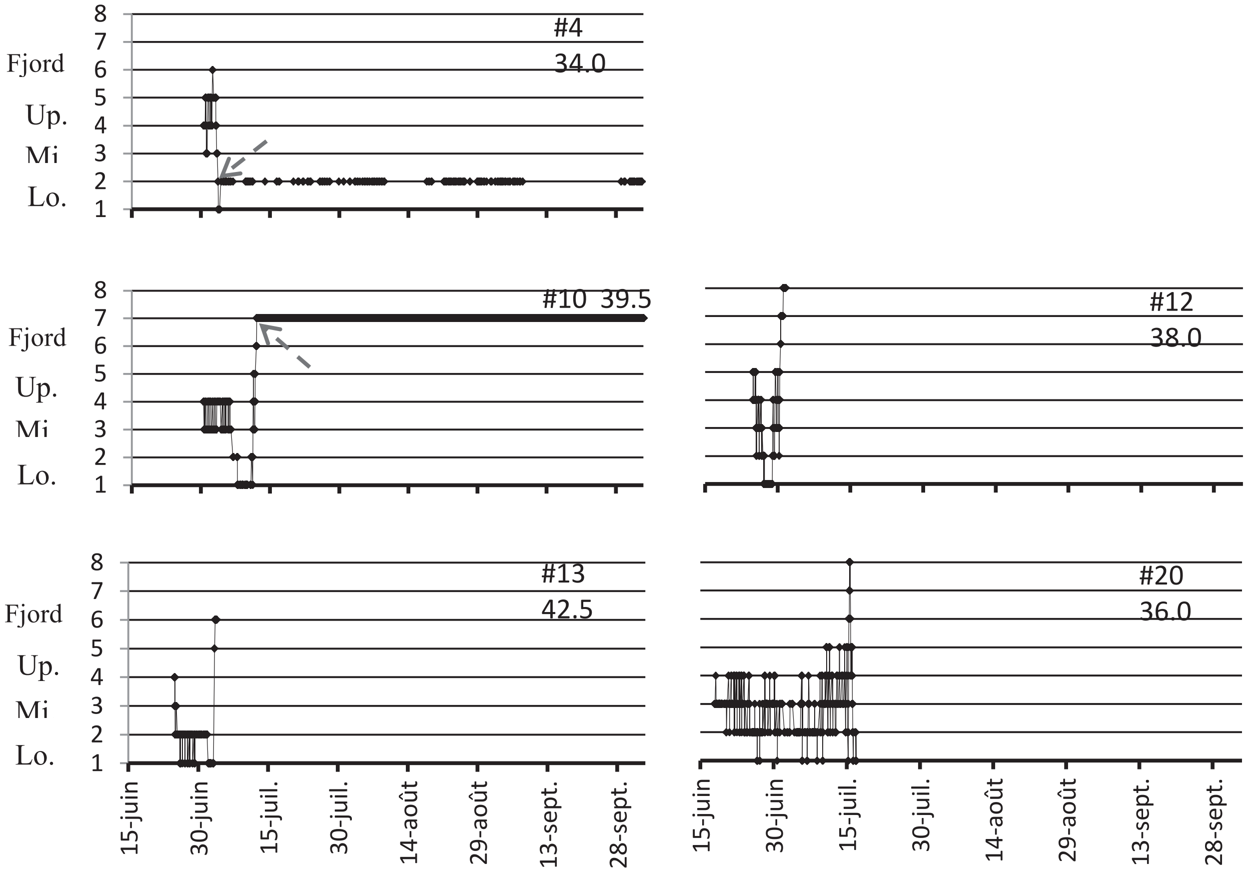

FIG. 5. Horizontal movement and habitat use of individual European whitefish in the estuary of River Neiden (p 20), and individuals that used both estuary and fjord (p 21). Fish identification number and fork length (cm) are given. The Y axes indicate the receiver numbers and the estuary zones; Upper (Up.), Middle (Mi.), Lower (Lo.).

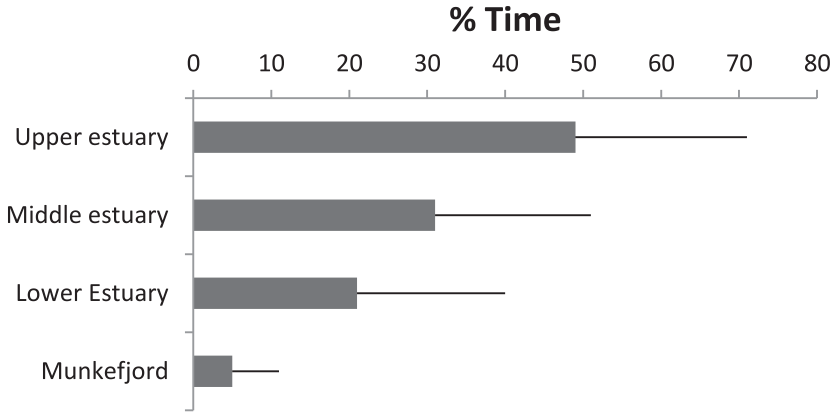

FIG. 6 Percentage of time spent by European whitefish at the different zones of the estuary and fjord from 16 June to 5 October. Error bars indicate the standard deviation.

Depth and temperature experienced by the fish

In all habitats of the estuary, the fish were mainly recorded at depth reflecting the mean depth of the habitat. In Munkefjord, fish spent > 50% of their time at ~1 m depth (mean = 1.4 , S.D. = 1.2) (Fig.7.a). In the upper and lower estuary, most depth record ranged between 2-3 m (mean upper = 2.2, S.D. = 0.83; mean lower = 2.4, S.D. = 0.99), 28% of the records in the lower estuary occurred between 3 and 6.4 m. In the mid-estuary, 48% of the records ranged between 1-2 m (mean = 1.8, SD = 0.8), which corresponds approximately to the average depth of this part. In every part of the estuary, the fish showed very little surface activity; registrations at a depth shallower than 0.5 m were rare (1%), while in Munkefjord 20 % of the fish records occurred 0.5 m deep.

Individuals were not detected in Munkefjord at temperature colder than 12 ˚C, and 77% of registration occurred at 12-14 ˚C (mean = 13.4; S.D. = 0.8). Individuals were recorded in the lower estuary at all range of detected temperatures between 5 and 20 ˚C, but with a slight peak at 12 – 14 ˚C with 26% of recording (mean = 13.5; SD = 2.8). Temperature recorded by the fish in the mid-estuary (mean = 13.1; SD = 3) were more variable. In the upper estuary 46% of record occurred between 14 and18 ˚C which is probably due to a more dominating river influx in this part of the estuary. The river had a higher temperature than the estuary and fjord during most of the study period.

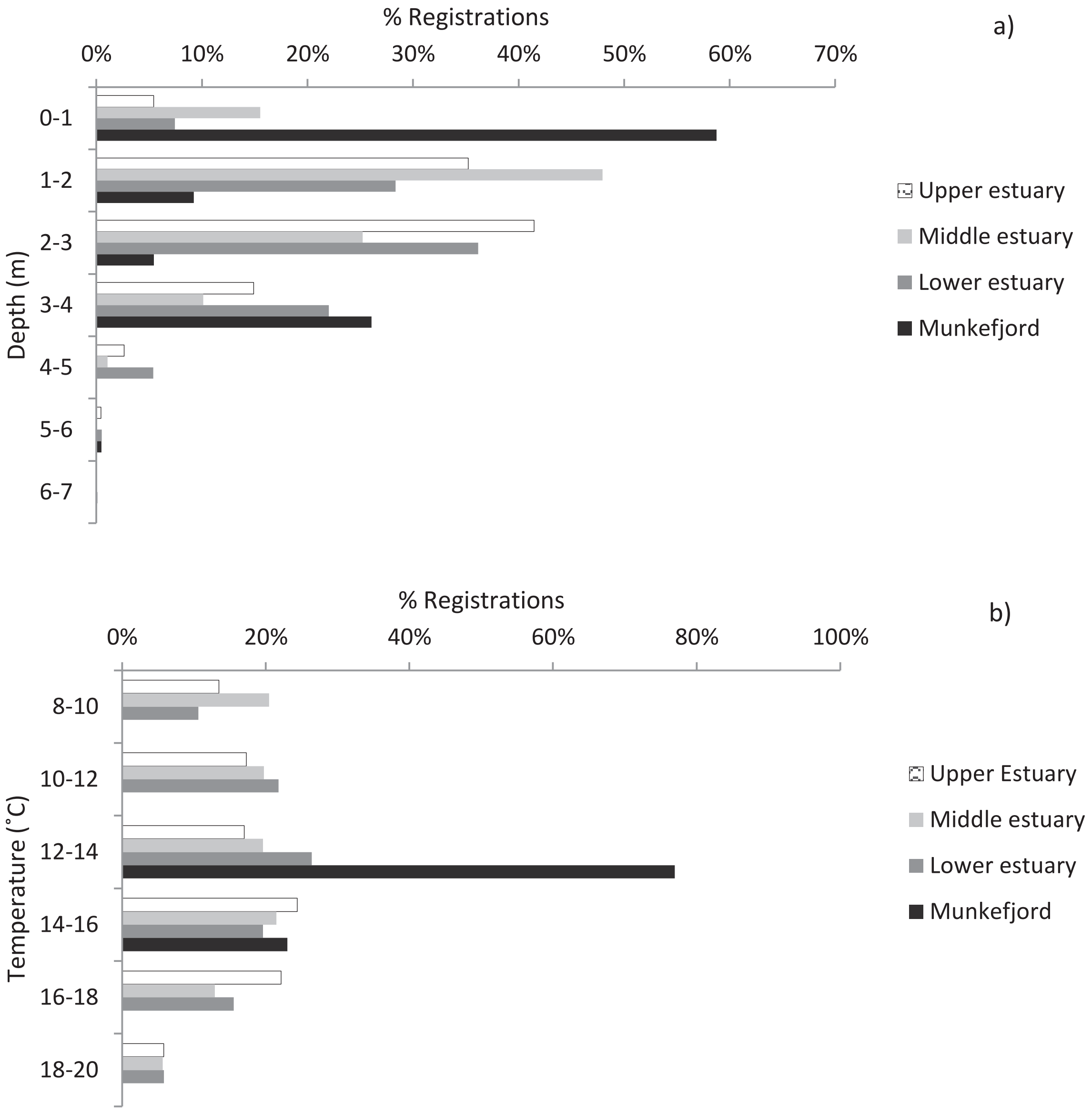

FIG 7. Percentage of registrations at different depths by acoustic transmitters implanted in European whitefish (a), and percentage of registrations at different temperatures (by intervals of 2 ˚C) (b), in each zone of the estuary and in the fjord.

Horizontal movement patterns in relation to tidal and salinity variation

Graphic plotting of the movement patterns against tidal or salinity variations, showed that most individuals (>80%) adopted a general movement pattern that followed the tidal cycle and the salinity concentration in the estuary habitats. The fish showed a tendency to retreat upstream from the lower estuary during high tide and elevated salinity, and return to this zone during low tide (Fig.8). At several occasions fish kept their position in the lower estuary during complete high tide prior to retreat upstream.

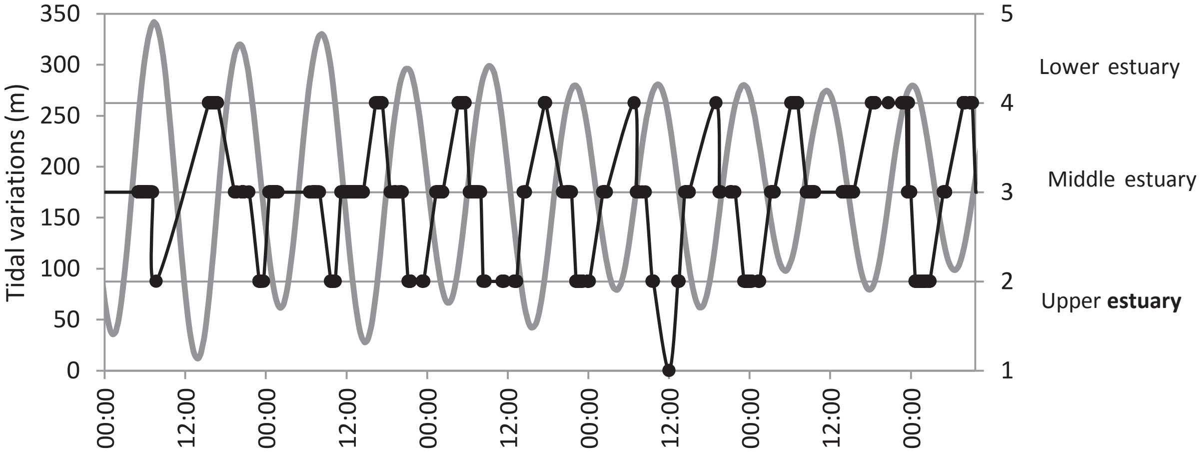

FIG. 8 Movements by one individual (#21) between the different zones of the estuary related to tidal fluctuations from 17 July 5:00 am until the 22 July 9:00 am. The dots indicate fish recordings by the different receivers.

To analyse if the salinity concentration in the estuary affected the habitat use of European whitefish; the fish records at a given zone (upper, middle, lower estuary) was compared with the tidal level at the time of individual record in a zone (Fig.9.a), and the salinity measurement (at the lower estuary) at the time of individual record in a zone (Fig 9.b). A significant difference was found between each zone for a given salinity; (Mann-Whitney *U*-test, $P < 0.001$) and for a given tidal level (Mann-Whitney *U*-test, $P < 0.001$). Still, 33% of the recordings in the lower estuary occurred at elevated salinity (> 15 ppt) during high tide (Fig. 9). However when examining the mean time spent by the fish in lower estuary, the fish showed a tendency to spend less time within this zone at higher salinity than at low salinity (Fig. 10). Considering that the P value has to be lower than 0.01 due to the Bonferroni correction for multiple comparisons; a significant difference was observed between the time spent at salinities < 15 ppt and the time spent at salinities within the range 25-30 ppt (Mann

Whithney *U*-test; P < 0.01)., no difference were found between the time spent at salinity < 15 ppt and the time spent at salinity between 15-20 ppt (P = 0.039) or between < 15 and 20-25 ppt (P = 0.014). There was no difference in time spent at salinity 15-20 ppt and 20-25 ppt (Mann Whitney *U*-test, P = 0.90). P was found to be low (P = 0.017) between 20-25 and 25-30 but not enough to reject the null hypothesis.

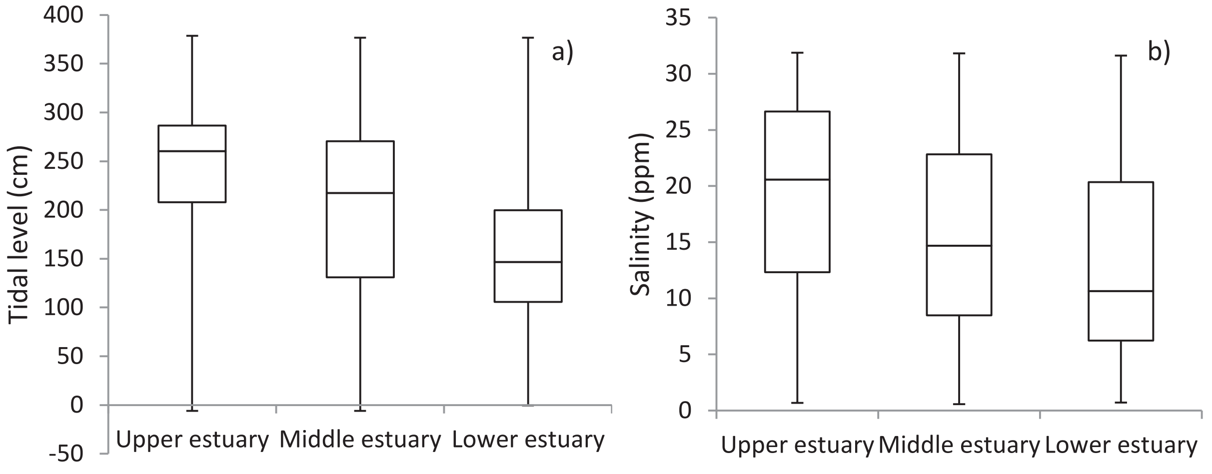

FIG. 9 Dispersion of fish records in the estuary zones for a given tidal level (a) and for a given salinity (measured in the lower estuary at 2 m depth) (b). Each box and error bars represent 25% of records, the line between two boxes gives the median.

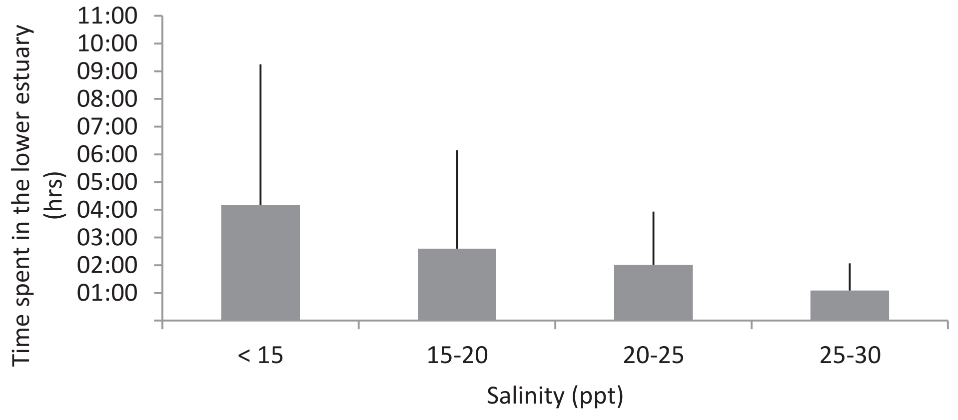

FIG. 10 Average time spent by tagged European whitefish in the lower estuary at salinity below and above 15 ppt, given at 5 ppt intervals for the values above 15 ppt. The error bars indicate the standard deviation.

The five fish recorded in Munkefjord, used this area for short time periods (mean: 3.20 hrs, S.D. = 4 min) and when the salinity of this area (measured at 2 m depth) was generally low (< 10 ppt). The exception was fish # 20 using the area for 8 hrs under a mean salinity of 20 ppt (mean depth; 1.73 m) and thereafter moved back to the upper estuary.

Vertical movement pattern and temperature in relation to salt water

When comparing the depth recorded by the fish to the salinity concentrations, fish seemed to keep utilizing the deeper zones at higher salinity without seeking the surface to reach the less salty layers (Fig.11). The fact that the fish were mainly registered below 2 m deep (mean 2.4 m) indicates that the salinity experienced by the fish was close to the one measured by the conductivity logger located *c*. 500 m downstream at 2 m depth.

As the river flow decreased and the water temperature increased, from the spring until the summer, both salinity and temperature increased in the estuary. Following this, the fish experienced the more elevated salinity concentrations at higher temperatures. The mean temperature experienced by the fish at salinities > 15 ppt was 15.4 ˚C (S.D. = 1.1).

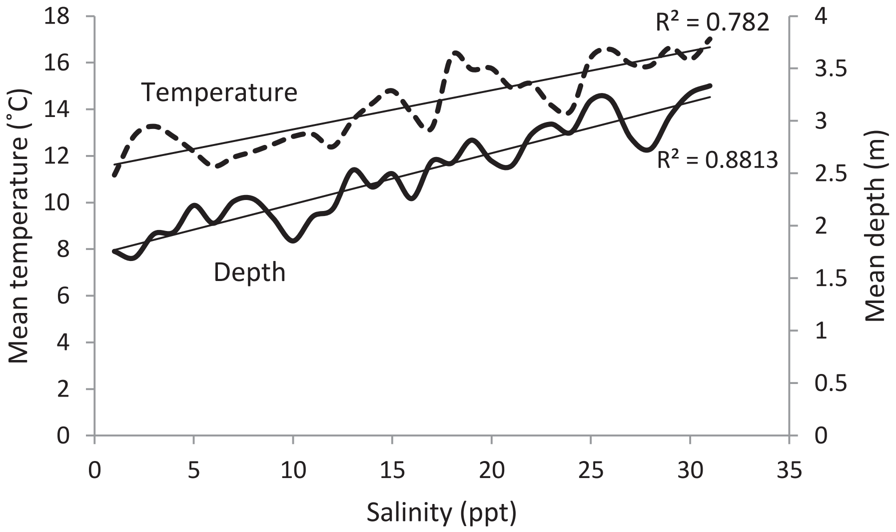

FIG. 11 Mean temperature (dotted line) and mean depth (filled line) sensed by individuals of tagged European whitefish in the lower estuary, given for the salinity recorded at 2 m depth in the lower estuary.

Physiological analyses and salt water challenge test

Gill Na$^+$, K$^+$-ATPase activity

European whitefish caught in the estuary of the River Neiden showed a generally low level of Na$^+$, K$^+$-ATPase activity, close to the level of fish residing in fresh water or slightly lower (92%, mean; 0.58, S.D. = 0.41 (Fig. 12). However, two individuals showed a highly elevated level; 3 and 9.9 µmol ADP mg prot.$^{-1}$, respectively, indicating that these fish might have stayed in salt water for some time period. The Na$^+$, K$^+$-ATPase activity of the tagged fish showed a low level among all individuals (mean: 0.6; S.D. 0.4).

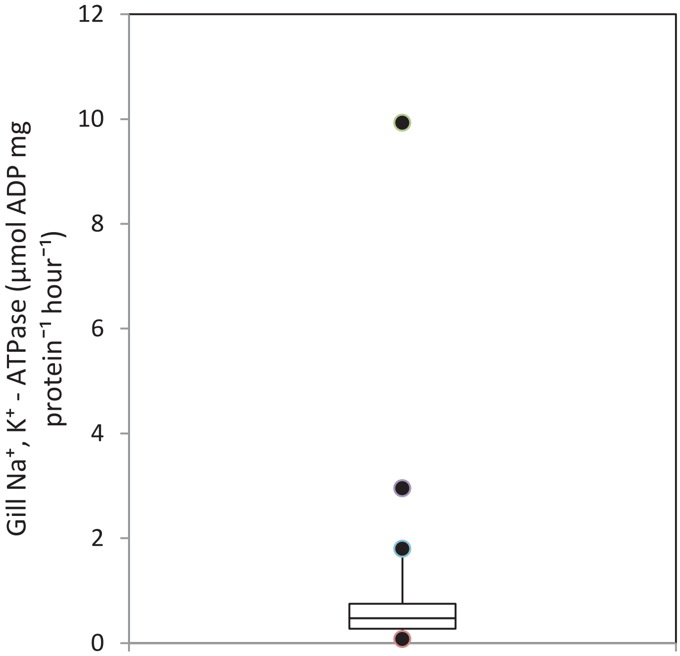

FIG. 12 Gill Na$^+$, K$^+$-ATPase activity of European whitefish caught in the estuary of the River Neiden between the 10 June and 10 July. The figure show both the ATPase activity of the fish sampled for physiological analyses (n = 11) and the tracked fish, (n = 19). Outliers are represented by black dots.

Salt water challenge

Five fish were transferred to natural sea water (30 ppt) at 10 ˚C during 24 hrs. Tree of the fish survived during the entire test period and two died *c.* 14 hrs ± 3 and 17 hrs ± 4 after the transfer. The osmolality of the fish that went through this test was much higher than the osmolality of the fish caught in the estuary during the same period (Fig. 13); mean S.W. test = 455.8 mmol kg^{-1}, S.D. = 29.2, mean estuary = 336.2 mmol kg^{-1}, S.D. = 30.2. One fish showed a level of ATPase activity (3 µmol ADP mg $prot.^{-1}$) higher than the level of the fish sampled in the estuary.

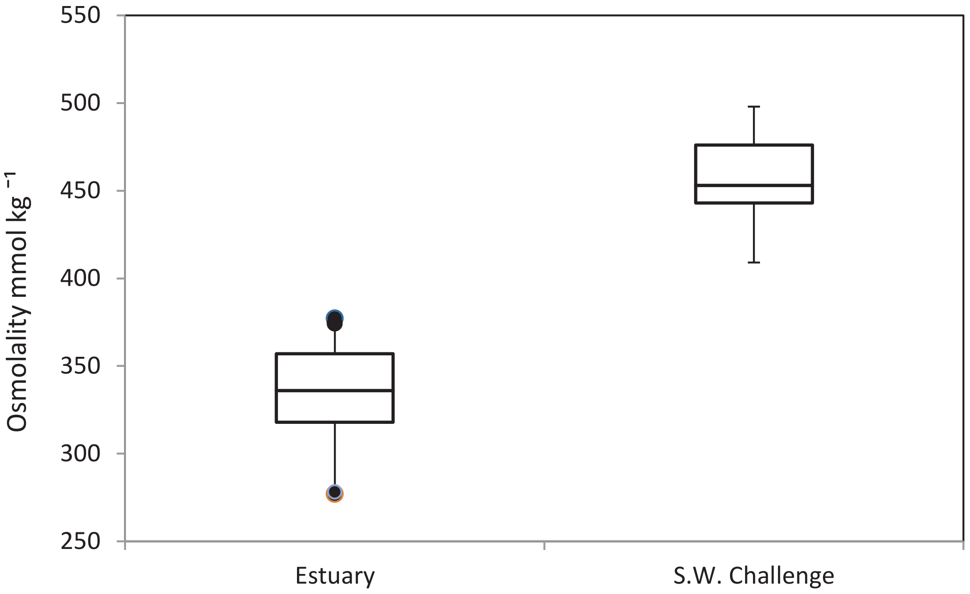

FIG. 13 Plasma osmolality of European whitefish caught in the estuary (n = 23) and European whitefish that went through a 24 hrs natural sea water (31 ppt) challenge test.

Discussion

General movement patterns and habitat choice

This study was the first to describe in detail the behaviour of European whitefish in an estuary by the use of acoustic telemetry. Earlier studies on anadromous European whitefish in brackish habitats have been conducted mainly by use of standard individual or group tags and recapture. These studies provided important information of for example; estimation of size of fish stock, habitat choice and some migration pattern, although mainly in the Baltic Sea, where the salinity is typically very low (< 10 ppt) (Leskelä 2006, Aronsuu & Huhmarniemi 2004, Lehtonen et al. 1992, Lehtonen & Himeberg 1992). They are, however, limited in that they only give information of the fish at tagging and recapture, while the electronic acoustic tagging gives the individual migration pattern more or less constantly. There is also very little documentation on riverine European whitefish having a short access to full strength sea water. However, Jonsson et al. (1988) used a standard tag-recapture-study to show a passive displacement to the sea under high river discharge, where only one individual was found at sea 100 m from the river outlet. The results from the lower River Neiden, provided by acoustic telemetry, showed that the European whitefish utilized actively the whole estuary area from spring to fall, following the tidal cycle, and sometimes also migrated through the fjord system to a neighbouring river.

Lehtonen & Himberg (1992) described the spawning migration of anadromous European whitefish in the Baltic Sea to occur between late June and October.
Ten of the European whitefish tagged in the estuary of River Neiden past the most upstream automatic receiver between the 16 July and the 12 August. These same ten fish were recorded by manual tracking the 5 and the 6 October at maximum 600 m interval downstream to the Waterfall Skoltefossen, in a putative spawning ground. These observations indicate that River Neiden European whitefish start their spawning migration between mid-July and August which confirmed the suggestion made by Staldvik (1989).

During the studied period seven individuals were missed from the detection range of the acoustic receivers. These fish could have expulsed their tag (Thorstad et al. 2013), the tag stopped working or the fish have died outside the detection range of the acoustic receivers. Another possibility could be that these fish resides actively outside of the detection range of any receivers. The distance between the last acoustic receiver at the River outlet and the first transect in Neidenfjord was 3.6 km, leaving a relatively large area without any possibility of acoustic reception. Most of the anadromous European whitefish in the Baltic Sea migrate from their home river and spend several years in more productive feeding areas before returning to their spawning river. There existed no data of the accurate salinity concentration in the inner Neidenfjord outside the estuary area, but the salinity measured at 2 m at the outlet of the River Neiden estuary indicates average of 31 ppt during the all summer, which is above the maximal salt water tolerance found for anadromous European whitefish (Madsen et al. 1996). The chance that these missing fish actively used this inner part of the Neidenfjord is therefore low. But since a large variability of salt water responses among populations of European whitefish was found (Papakostas et al. 2012), and that the salt water tolerance of this particular population has not been completely tested, we cannot reject the theory that they have been outside the detection area.

Habitat selection and salt water tolerance

The habitat selection of European whitefish in this study was correlated with the salt water influx that followed the tide cycle. The main behavioural pattern was identified as a horizontal retreat from the lower part of the estuary under high tide and inflow of sea water with high salinity concentration. When the individuals were exposed to high salt water concentration in the lower estuary, their residency in these areas was generally found to be of short duration, often not exceeding one hour, followed by a retreat upstream to less saltier water.

In order to be able to tolerate high salt water concentration over long periods, the fish have to develop hypo-osmoregulatory physiological mechanisms, often identified by an increased level of gill-ATPase activity (McCormick et al. 2009). But, since European whitefish does not seem to be able to develop this mechanism prior to a transfer in salt water (Madsen et al. 1996), it implies that this species has to cope with a consequent physiological stress when occurring in salt water; including muscle tissue dehydration, red blood cell shrinkage or a decrease in respiratory abilities, before a possible increased ATPase can be initiated to regulate this salinity stress to a normal level (Madsen et al. 1996). In contrast to laboratory experiments where the fish are constrained in a tank filled with sea water, the estuary of the River Neiden gives the European whitefish the possibility to move in and out of salt water, as the estuary area is only about 6 km and often highly stratified with the possibility to find vertical and horizontal decreasing salinity gradients. The highest salt water concentration in the estuary occurred during summer at lower river discharges and when water temperatures where higher, which may increase the physiological salt tolerance of European whitefish (Finstad et al. 1988, Handeland et al. 1998). The River Neiden European whitefish thus seems to adopt a behavioural strategy, that consist of using the lower estuary to feed on more abundant marine prey (Ysebaert et al. 1993, Josefson & Hansen 2004) and move horizontally, and positively also vertically between fresh and salt water border.

Staldvik (1989) studied the diet of European whitefish caught in the River Neiden, in the estuary and in Munkefjord. A comparison of the stomach contents showed that fish caught in the estuary and in the fjord fed mainly on marine preys like Gammaridae and Mysidae, and some Euphausiacea (krill). I contrast, most of these preys were not found in the stomachs of the European whitefish captured in the river, except for a few Gammaridae. The stomach fullness (% filling) of the fish sampled in the river was only half that of the fish caught in the estuary and fjord. As Gammaridae are benthic prey items and these largely dominated the stomach content of the fish caught in the estuary (Staldvik 1989), this correspond to our data from acoustic telemetry that showed that the European whitefish in River Neiden were almost exclusively using the deeper water in the estuary, probably mostly and under the halocline (the border between fresh and salt water). The small test done in this study were a few fish were kept in a cage in salt water, indicated that at least some individuals were able to survive at high salt water salinity for up to 24 hours. This indicates that the European whitefish are able to actively feed in the estuary for shorter periods under high salt water concentration. During the gillnet sampling of European whitefish from 16 June to 3 July in estuary, no exclusive salt water fish species were caught. The only other fish species that were caught were adult Atlantic salmon, anadromous brown trout and the catadromous European flounder. As Atlantic salmon usually does not feed during its upstream migration, the only potential fish competitors of European whitefish in the estuary of River Neiden are brown trout and European flounder, although this is difficult to verify.

Risk of predation has been mentioned as a limiting factor for freshwater fish to use the estuary habitat, and especially in the lower reaches where the number of marine predators is assumed to be higher (Whitfield 2015). Such predation risk could also be a limiting factor for juveniles drifting in the lower part of the estuary (Lehtonen et al. 1992, Næsje et al. 1986). Considering large sized individuals used in this study, predation by marine fish is likely to not be a limiting factor. But since a large colony of harbour seals (*Phoca vitulina*) resides in the estuary and in Munkefjord, we could expect a relatively high degree of predation by seals on European whitefish especially since these fish seemed to be using the uppermost water column in this area. However, none of the fish that were potentially dead (see earlier discussion) showed a significantly increase in temperature that should be expected if the tag were swallowed by a warm blooded predator, although the tag could also been lost during predation and not swallowed.

Gill Na^+, K^+ ATPase activity and individual variation

Most of the sampled and tagged fish in this study showed a low level gill ATPase activity, close to the levels observed for European whitefish in fresh water (Madsen et al. 1996). However, two individuals showed a remarkably high level compare to the rest of the sample, and one individual had a level of gill ATPase activity close normally observed for brown trout post-smolts (Aarestrup et al. 2000). Even if this only constitute two observations on a low sample size ($n = 30$), this indicates a certain individual variability among the River Neiden European whitefish in the acquisition of hypo-osmoregulatory mechanisms. Local fishermen mentioned that they had on some occasion caught a European whitefish in gillnets in the Neidenfjord area up to10 km away from the River Neiden outlet (personal information Knut Skimlid). River Neiden is the only river of the fjord area where European whitefish have been documented. The high ATPase-levels in a few fish and the possible capture of a European whitefish far away from the River Neiden estuary may therefore indicate that some individuals from the River Neiden population might be able to develop a sufficient hypo-osmoregulatory ability to cope with higher level of salinity concentration and therefore move further away from the estuary area.

Conclusions

In sum, this study demonstrated that European whitefish in the lower Neiden watercourse utilize the estuary extensively as a feeding area. The results indicated that at least a few individuals could possibly cope with relatively high salinity concentration over some time. However, as most of the European whitefish was only recorded to stay in salt water for shorter periods, and only relatively close to the estuary areas, these fish may not be regarded as truly anadromous in the sense that they do not stay for longer periods in pure salt water. Thereby they seem to rather have adapted a behavioural strategy to make them able to utilize the surplus of marine and energy rich prey that seemingly aggregates in this habitat, thereby also reducing potential competition with their conspecific or other fish species in pure fresh water.

Further studies should focus on studying their diet in more detail, including isotope analysis in order to quantitatively measure the importance of marine prey items in their diet over time. Also, genetic and physiologic studies should be conducted on European whitefish below and above the Waterfall Skoltefossen to test if the European whitefish below the waterfall potentially originate from European whitefish larvae that have drifted down the waterfall, or if it is a separate population that needs to be given a special emphasis in the management of European whitefish.

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