Effects of hatchery rearing practices and cortisol manipulation on growth, survival and seaward migration success of stocked and wild Atlantic salmon and brown trout smolts

PhD thesis by
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Technical University of Denmark
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PREFACE

This thesis is submitted in partial fulfillment of the requirements for obtaining the degree of Doctor of Philosophy (PhD) at the Technical University of Denmark (DTU). It represents work conducted between December 2011 and May 2015, primarily at the National Institute for Aquatic Resources (DTU Aqua), Section for Freshwater Fisheries and Ecology in Silkeborg. The presented research was conducted as part of the strategic project SMOLTPRO, financed by the Swedish Research Council Formas. The overall goals of SMOLTPRO are to develop ecologically and ethically sound methods for supplementary rearing of salmonids and to increase the socioeconomic returns of hatchery-produced Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) in Scandinavia.

This project would not have been possible without the guidance, contributions, and support from many people. First and foremost, I want to thank my main supervisor Kim Aarestrup for the opportunities I have been given. Thanks for your inspiration, encouragement, and never-ending support through the years. It has been a great pleasure to work with you. Likewise, I am deeply grateful to my co-supervisors Christian Skov and Erik Höglund for insightful comments, constructive criticism, and support.

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Silkeborg, May 2015

Martin Hage Larsen

Martin Hage Larsen

LIST OF PAPERS

This thesis is based on the work presented in the following papers, referred to in the text by their respective roman numeral:

I. Midwood, J.D., **Larsen, M.H.**, Boel, M., Jepsen, N., Aarestrup, K., Cooke, S.J. (2014). Does cortisol manipulation influence outmigration behaviour, survival and growth of sea trout? A field test of carryover effects in wild fish. Marine Ecology Progress Series. 496: 135–144. doi: 10.3354/meps10524.

II. Midwood, J.D., **Larsen, M.H.**, Boel, M., Aarestrup, K., Cooke, S.J. An experimental field evaluation of winter carryover effects in semi-anadromous brown trout (*Salmo trutta*). Journal of Experimental Zoology Part A: Ecological Genetics and Physiology. Submitted.

III. **Larsen, M.H.**, Johnsson, J.I., Näslund, J., Thomassen, S.T., Aarestrup, K. Reduced rearing density increases post-release migration success of Atlantic salmon (*Salmo salar*) smolts. Canadian Journal of Fisheries and Aquatic Sciences. Submitted.

IV. **Larsen, M.H.**, Johnsson, J.I., Winberg, S., Wilson, A.D.M., Hammenstig, D., Thörnqvist, P-O., Midwood, J.D., Aarestrup, K., Höglund, E. (2015). Effects of emergence time and early social rearing environment on behaviour of Atlantic salmon: consequences for juvenile fitness and smolt migration. PLOS ONE. 10(3):e0119127. doi: 10.1371/journal.pone.0119127.

V. **Larsen, M.H.**, Thorn, A.N., Skov, C., Aarestrup, K. (2013). Effects of passive integrated transponder tags on survival and growth of juvenile Atlantic salmon *Salmo salar*. Animal Biotelemetry. 1:19. doi: 10.1186/2050-3385-1-19.

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SUMMARY

Anadromous Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) spend their early life stages in freshwater habitats before they transform into smolts and migrate to the ocean to feed and grow. The process of smoltification, which pre-adapts juveniles to live in saltwater environments, is a challenging and energy-demanding period involving changes in behavior, morphology, and physiology. At the same time, smolts are often exposed to high levels of predation along their downstream migratory route to the sea. These circumstances make the pre-smolt and smolt life stages especially sensitive to external perturbations, yet very little is known about the response of wild salmonids populations to stressors in their natural environment.

This thesis includes two complementary studies that aimed to investigate the effects of prolonged stress on growth, survival, and seaward migration success of juvenile brown trout by artificially increasing blood plasma cortisol (stress hormone) levels with intra-coelomic injection of cortisol suspended in molten cocoa butter. This technique is an increasingly common approach to mimic the physiological effects of exposure to natural and anthropogenic stressors in teleost fishes. In the first study (**paper I**), we manipulated cortisol titers of brown trout in a natural stream in early spring, immediately prior to the typical smolt migration period. Our results show that the seaward migration success of trout that received a high dose of cortisol was reduced relative to a control group. Cortisol-treated individuals remaining in the stream following the smolt run also exhibited lower growth rate. In the second study (**paper II**), plasma cortisol titers of the trout were artificially raised in late autumn. The overwinter mortality was 2.5 times higher for trout experiencing chronic cortisol elevations when compared to individuals in a control group. The downstream migration success of chronically stressed trout was also less than half than that of control individuals during the winter and spring. In addition, while control fish continued to grow over the winter, cortisol-treated individuals showed negative growth. Taken together, these studies suggest that chronic elevations of cortisol can reduce survival of juvenile brown trout, negatively impact growth of fish that survive, and ultimately result in a reduction in the number of migratory trout. Over a longer timescale, there is therefore large potential for a stressor to affect the structure and viability of wild salmonid populations.

Many Atlantic salmon and brown trout populations have been lost or experienced severe declines across their natural distribution owing to anthropogenic factors, such as habitat destruction, pollution, damming of rivers, and overexploitation. To compensate for these declines, large numbers of hatchery-reared salmon and trout are annually stocked into nature through supplementation programs in several nations. However, because the post-release survival of hatchery-reared salmonids is usually considerably lower than that of their wild counterparts, there is currently growing interest in developing and improving rearing methods to increase performance of hatchery fish following release into nature. In **paper III**, we investigate the effect of hatchery rearing density on the post-release survival of one-year old Atlantic salmon smolts during the riverine migration to the sea. Fish were either reared at conventional hatchery density (1,500 fish m^{-2}) or at one third of conventional density (500 fish m^{-2}). The results show that dorsal fin and opercula damage were more severe among fish reared at conventional density. In the stream, the seaward migration success was 16% higher for Atlantic salmon reared at reduced density than for individuals kept at conventional density. These novel results emphasize that there is scope for improving rearing methods of supplementation and/or conservation hatcheries in order to increase welfare in rearing and optimize outcome of releases.

In salmonid fishes, individual variation in timing of spawning nest emergence, when fry establish a territory and commence exogenous feeding, appear to be linked to behavioral and physiological traits as well as performance under hatchery conditions. Therefore, the underlying question of **paper IV** was whether sorting Atlantic salmon according to larval development rate in the hatchery offers a possibility to select fish with traits especially suitable for restocking. In contrast to previous studies, we only found little evidence that time to emergence from artificial spawning nests was correlated with individual behavioral expression. Moreover, emergence time was not related to post-release growth, survival, or migration status of juvenile Atlantic salmon in a near-natural experimental stream. From an applied perspective, these findings highlight the challenges associated with incorporating individual variation in larval development rate into fisheries management and conservation.

Passive integrated transponder (PIT) telemetry was a critical component of this thesis as it allowed us to monitor the individual growth, survival, and migration behavior of several hundred Atlantic salmon and brown trout. An underlying assumption of telemetry studies is that the tagging procedure and tag itself do not adversely affect the tagged individual. Hence, researchers often have to balance the trade-off between PIT tag size and detection range, especially in studies involving smaller bodied fish species. In **paper V**, we evaluated the effects of surgically implanted 23 and 32 mm PIT tags on growth, survival, and tag retention of Atlantic salmon 80 to 135 mm fork length (FL) in a laboratory environment. Our study suggests that marking juvenile Atlantic salmon with 23 mm PIT tags result in high retention rate (≥97%), regardless of whether tagging incisions are closed with absorbable suture or not. However, we caution against the use of suture due to high rates (35%) of fungal infection and tissue inflammation around the incision site. When leaving the incision non-sutured, results indicate that 23 mm PIT-tagging of Atlantic salmon 100 to 135 mm FL is feasible because it does not adversely affect growth and survival. By contrast, we do not recommend fisheries managers and researchers to use 32 mm PIT tags as an individual marker in juvenile Atlantic salmon due to high rate of tag rejection (69%) and reduced growth and survival of the tagged individuals.

Overall, this thesis contributes to our understanding of the ecology of stress in wild salmonid populations. Specifically, the results suggest that chronic cortisol elevations may have severe impacts on wild brown trout populations by reducing the number of smolt that successfully enters the sea. The thesis also shows that there is scope for improving rearing methods of supplementation hatcheries to increase the stocking success of hatchery-reared Atlantic salmon. In particular, rearing at reduced density seems to be a promising approach to improve the survival of smolts during seaward migration. Lastly, this thesis has provided some recommendations for PIT-tagging juvenile Atlantic salmon that should be useful for future monitoring studies.

DANSK RESUMÉ

De tidlige livsstadier af anadrome atlantiske laks (*Salmo salar*) og ørreder (*Salmo trutta*) foregår i ferskvand, inden de udvikler sig til smolt og vandrer mod havet for at udnytte de større føderessourcer. Smoltifikationen, som tilpasser de juvenile fisk til at leve i saltvand, er en udfordrende og energikrævende process der involverer en række omfattende ændringer i fiskens adfærd, morfologi og fysiologi. Samtidig er smolt ofte udsat for et høj prædationstryk under deres nedstrøms vandring til havet. Derfor er smolt stadiet en kritisk periode, der er særlig følsom over for udefrakommende forstyrrelser. På trods af disse omstændigheder findes der kun ganske få studier, der har undersøgt, hvordan miljømæssige stressfaktorer påvirker vilde bestande af laks og ørred.

Denne afhandling indeholder to komplementære studier, der har til formål at undersøge, hvordan kronisk stress påvirker vækst, overlevelse og vandringsædfærd hos juvenile ørreder i deres naturlige miljø. Dette blev belyst ved at injicere kortisol (stresshormon) i fiskens bughule ved injektion med sprøjte. Injektion af kortisol er en anerkendt metode til at simulere naturlige og antropogeniske stresspåvirkninger hos fisk, og den mængde der blev brugt svarer til naturligt forekommende kortisol niveauer hos ørred. I den første undersøgelse (**artikel I**) blev ørredernes blodplasma kortisol niveau øget i det tidlige forår i et naturligt vandløb umiddelbart før den typiske vandringsperiode til havet. Resultaterne viser, at vandringssuccesen til havet for ørreder der blev behandlet med en høj dosis af kortisol var reduceret i forhold til en kontrolgruppe af fisk. Kortisol-behandlede individer der ikke vandrede til havet havde også lavere vækstrate. I den anden undersøgelse (**artikel II**) blev ørrederne behandlet med kortisol i det sene efterår. Dødeligheden over vinteren var 2,5 gange højere for kortisol-behandlede ørreder sammenlignet med individer i en kontrolgruppe. Vandringssuccen til havet var også halveret for de stressede ørreder. Derudover var væksten lavere for de kortisol-behandlede ørreder over vinteren. Tilsammen viser disse undersøgelser, at forhøjet blodplasma kortisol niveau over en længere periode kan reducere overlevelsen af juvenile ørreder, negativt påvirke væksten hos overlevende fisk og i sidste ende føre til en reduktion i antallet af vandrende smolt. Over en længere tidshorisont kan langvarig stress derfor få alvorlige konsekvenser for strukturen af vilde ørred populationer.

Mange oprindelige bestande af laks og ørreder er helt eller delvis blevet udryddet på tværs af deres naturlige udbredelsesområder på grund af antropogeniske faktorer, herunder ødelæggelse af levesteder, forurening, opdæmning af floder og overudnyttelse. For at kompensere for disse bestandsreduktioner udsættes et stort antal opdrættede laks og ørreder i mange lande. Overlevelsen af opdrættede laksefisk er dog ofte lav i forhold til vilde individer i naturen. Der er derfor et stigende behov for at udvikle og forbedre opdrætsmetoder, der øger fiskenes overlevelse efter udsætningen. Formålet med **artikel III** var, at undersøge om en reduktion i opdrætstæthenden af fisk i konventionelle kækkerier kan forbedre overlevelsen af laksesmolt efter udsætning. Fiskene blev opdrættet ved konventionel tæthed (1500 fisk m^{-2}) eller en tredjedel af konventionel tæthed (500 fisk m^{-2}). Resultaterne viser, at skader af rygfinne og gællelåg var mere udbredt hos fisk ved konventionel opdrætstæthed sammenlignet med fisk, der blev opdrættet ved den lavere tæthed. I et naturligt vandløb var vandringssuccen til havet 16 % højere for laksesmolt, der blev opdrættet ved reduceret tæthed. Disse resultater understreger, at der er stort potentiale for at optimere konventionelle opdrætsmetoder i forhold til at forbedre kvaliteten og overlevelsen af udsætningsfisk i naturen.

Laks og ørred lægger deres æg i grus på bunden af vandløb. Efter klækningen ligger larverne passivt mellem gydegruset, mens de bliver ernæret af blommesækken. Når blommesækken er ved at være opbrugt, begynder larverne at bevæge sig op gennem gruset for at etablere et territorium og aktivt opsøge føde. Tidligere undersøgelser har vist, at den individuelle variation i timingen af denne vandring fra gydegruset er koblet til både adfærdsmæssige og fysiologiske træk. Det er også blevet foreslået, at der er en sammenhæng mellem larvernes udviklingsrate og deres fremtidige vækst samt sandsynligheden for at smoltificere som etårige fisk. I **artikel IV** undersøgte vi, om sortering af lakse larver i forhold til deres vandring fra kunstige gydereder i klækkeriet kan udnyttes til at udvælge fisk med bestemte træk der er særligt velegnede til udsætninger. I modsætning til tidligere undersøgelser viser resultaterne kun en svag sammenhæng mellem vandringstidspunktet fra de kunstige gydereder og fiskens adfærd. Derudover var timingen af ynglens vandring fra gyderederne ikke relateret til væksten, overlevelsen eller andelen af fisk der smoltificerede som etårige i et semi-naturligt vandløb. Fra et anvendt perspektiv fremhæver vores resultater nogle af de udfordringer der er forbundet med at inkorporere individuel variation i adfærd og udviklingsrate af fiskelarver i forvaltningen af bestande af laks.

PIT (passive integrated transponder) telemetri var et vigtigt redskab i denne afhandling. Denne teknologi muliggjorde at monitere vækst, overlevelse og vandringsadfærd hos flere hundrede laks og ørreder. Den underliggende antagelse i telemetri studier er, at mærkningsproceduren og mærket ikke har betydelig negativ indflydelse på de mærkede individer. PIT-mærker findes fra 8 til 32 mm i længde. De små mærker (8 til 12 mm) er velegnet til mindre fisk, men desværre er detektion rækkevidden også lavere for disse mærkestørrelser i forhold til større mærker (23 til 32 mm). Formålet med **artikel V** var, at undersøge hvorvidt mærkning med 23 og 32 mm PIT-mærker påvirker væksten og overlevelsen af laks mellem 80 og 135 mm fork længde (FL) i et laboratorieforsøg. PIT-mærkerne blev implanteret i fiskenes bughule gennem et 3-4 mm langt snit i bugvæggen. Resultaterne viser, at mærkning med 23 mm PIT-mærker er forbundet med et lille mærketab (≤3 %), uanset om snittet lukkes med absorberbar sutur eller efterlades åbent. Vi anbefaler dog ikke at snittet lukkes med sutur, fordi denne metode var forbundet med en høj prevalæns (35 %) af svampeinfektion og inflammation omkring snittet. Idet mærkning med 23 mm PIT-mærker uden sutur ikke påvirkede vækst og overlevelse hos laks mellem 100 og135 mm FL, er denne mærkningsmetode egnet til individuel mærkning af juvenile laks Vi anbefaler derimod ikke, at juvenile laks mærkes med 32 mm PIT-mærker, da disse reducerede fiskenes vækst og overlevelse. Desuden var mærkningen med 32 mm PIT-mærker forbundet med et højt mærketab (69 %).

Resultaterne fra denne afhandling bidrager til vores forståelse af hvordan stress kan påvirke vilde ørred bestande. Vores undersøgelser tyder på, at kronisk forøgelse af kortisol niveauer i de tidlige livsstadier kan reducere smoltproduktionen og dermed bestanden af vilde ørreder. Endvidere viser resultaterne fra denne afhandling, at der er mulighed for at forbedre eksisterende opdrætsmetoder med henblik på at øge udbyttet af udsætningerne. Herunder tyder resultaterne særligt på, at en reduktion i antallet af individer i opdrætstankene er et lovende tiltag i forhold til at øge overlevelsen af opdrættede laks i naturen. Endelig har denne afhandling givet nogle anbefalinger til brugen af PIT-mærker i juvenile laks, som er nyttige for fremtidige undersøgelser.

OBJECTIVES

This thesis consists of five scientific papers that fall under three distinct themes: (1) impacts of stress on juvenile salmonids, (2) stocking of hatchery-reared salmonids as a management tool, and (3) use of PIT telemetry to study juvenile salmonids. The objectives of the first two papers (**I** and **II**) were to gain insight into the consequences of stress on growth, survival, and seaward migration success of wild brown trout by artificially increasing blood plasma cortisol levels using exogenous cortisol implants (theme 1). The next two papers (**III** and **IV**) focused on developing rearing methods that improve the stocking success of hatchery-reared Atlantic salmon (theme 2). For this work, we examined the effect of hatchery rearing density on the post-release migration success of Atlantic salmon smolts (**paper III**). We further investigated whether larval developmental rate and individual difference in behavior of hatchery-raised Atlantic salmon influence growth, survival, and smolt migration following release into nature (**paper IV**). The purpose of the fifth and final paper (**V**) was to determine the feasibility of PIT-tagging juvenile salmonids as this technique was a critical tool in the thesis (theme 3). As set out below, the specific objectives of this thesis were to:

- Elucidate whether and to what extend exposure to chronic cortisol elevation during early spring affect growth, survival, and seaward migration success of juvenile brown trout (**paper I**).
- Assess the impacts of chronic cortisol elevations during late fall on the overwinter growth and survival of juvenile brown trout. The consequences of elevated blood plasma cortisol levels on the riverine migration success of trout to the sea are also examined (**paper II**).
- Investigate whether hatchery rearing density influence the post-release migration success of one-year old Atlantic salmon smolt to the sea (**paper III**).
- Examine the effects of variation in timing of spawning nest emergence and social rearing environment on individual behavioral expression and the potential consequences for the post-release growth, survival, and smolt migration of Atlantic salmon (**paper IV**).
- Evaluate the effects of 23 and 32 mm PIT tags on growth, survival, and tag retention of juvenile Atlantic salmon (**paper V**).

The present thesis opens with a general introduction and synopsis that outline the framework for the research, summarizes and discusses the key findings of **papers I** to **V**, and provides requirements and directions for future research.

INTRODUCTION

The general introduction is organized into five sections. The first section provides a brief outline of the natural distribution, life history strategies, and life cycle of Atlantic salmon and brown trout. Section two briefly describes the smoltification process and smolt behavior during the downstream migration to the sea. Important natural and anthropogenic factors that have potential to decrease the seaward migration success of smolts are also discussed. This theme is continued in section three with an examination of the potential impacts of chronic cortisol elevations on the riverine migration success of smolts to saltwater environments (theme 1). Section four focuses on stocking of hatchery-reared parr and smolts as a management tool to compensate for declines in natural production (theme 2). Specifically, it stresses the need to improve and develop rearing methods in supplementation hatcheries that increase the stocking success of hatchery-reared salmonids. The fifth and final section evaluates different methods for studying smolt migration and survival with focus on passive integrated transponder (PIT) telemetry (theme 3).

1. ATLANTIC SALMON AND BROWN TROUT

Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) both belong to the genus *Salmo*. Both species are of high social and economic importance, through recreational fisheries, commercial fisheries, and aquaculture (Harris and Milner 2006, Jonsson and Jonsson 2011, ICES 2013). Atlantic salmon and brown trout exploit freshwater and saltwater, and have adapted to feed in streams, rivers, lakes, estuaries, and the open ocean. Populations may become resident in freshwater and spend their entire life in local restricted areas or migrate several thousand kilometers to the ocean for feeding before returning to the natal river for spawning. For the purposes of this thesis, I adopt Northcote's (1978) definition of fish migration: "*movements that result in an alternation between two or more separate habitats, occur with a regular periodicity, and involve a large part of the population*". This section provides a brief review of the geographical distribution, life history strategies, and life cycle of Atlantic salmon and brown trout.

1.1. Geographical distribution

The Atlantic salmon is naturally distributed along the east and west coasts of the North Atlantic Ocean. On the west side of the Atlantic Ocean, salmon occur in several rivers from New England in the United States in the south to Ungava Bay in Canada in the north. A local population of Atlantic salmon is also found in the Kapisillit River on the west coast of Greenland close to Nuuk. On the east side of the Atlantic Ocean, salmon are distributed from River Minho in northern Portugal to rivers draining into the Barents and White Sea areas of northwestern Russia (MacCrimmon and Gots 1979, Jonsson and Jonsson 2011, Thorstad et al. 2011a). Atlantic salmon also occur naturally in several rivers draining into the Baltic Sea (MacCrimmon and Gots 1979, Jonsson and Jonsson 2011). In addition, its natural distribution includes several rivers of the United Kingdom, Iceland, and Ireland. Atlantic salmon feed over large areas in the North Atlantic Ocean, occasionally migrating as far north as Svalbard (81°N; Jonsson and Jonsson 2011).

The native distribution of brown trout is chiefly restricted to Europe (MacCrimmon and Marshall 1968, MacCrimmon et al. 1970, Klemetsen et al. 2003). The northern limits of its natural distribution extend from Iceland eastwards to northern Norway and northwestern Russia, while the southern limits are the northern coastline of the Mediterranean Sea and the Atlas Mountains of North Africa. The western limits are defined by the European coastline and the eastern limits are probably the Ural Mountains in the north and the Amu Darya River drainage of the Aral Sea in the south (MacCrimmon and Marshall 1968). Although brown trout is indigenous to the northern hemisphere, its present distributions are larger and widely influenced by human activities, such as aquaculture and recreational fisheries (MacCrimmon and Marshall 1968, MacCrimmon et al. 1970). As a result of introductions outside its natural range, the distribution of brown trout now includes areas of North and South America, Japan, New Zealand, Australia, and South and East Africa (MacCrimmon et al. 1970).

1.2. Life history strategies

Atlantic salmon and brown trout exhibit a complex and diverse array of life-history strategies. Most populations of Atlantic salmon are anadromous, spending much of their lives feeding in the ocean before returning to their natal river for reproduction. However, non-anadromous Atlantic salmon populations are known from a number of localities, such as River Namsen and Lake Brylandsfjord in Norway, Lake Vänern in Sweden, Lake Saimaa in Finland, and Lake Ontario in Canada (Jonsson and Jonsson 2011). Non-anadromous Atlantic salmon populations complete their entire life cycle in freshwater, typically utilizing lakes as feeding area instead of the ocean (Berg 1953, Jonsson and Jonsson 2011, Thorstad et al. 2011a). Few populations, however, spend their entire life cycle in rivers without migrating to lakes or seawater for feeding (Berg 1953, Thorstad et al. 2011a).

Like Atlantic salmon, brown trout form both anadromous and non-anadromous populations. Individuals in non-anadromous brown trout populations may migrate between different freshwater habitats, such as rivers and lakes, or complete their life cycle in rivers as resident individuals (Jonsson and Jonsson 2011). Anadromous brown trout, usually termed sea trout, spawn in freshwater and migrate to marine habitats for feeding. They predominantly feed in estuaries and along the coast, and are seldom found offshore in the open ocean like Atlantic salmon (Klemetsen et al. 2003, Jonsson and Jonsson 2011). Anadromous brown trout often exhibit partial migration with a portion of the population in a system becoming resident and the remaining individuals performing seasonal migrations between freshwater and seawater habitats (Elliott 1994, Klemetsen et al. 2003, Olsson and Greenberg 2004, Jonsson and Jonsson 2011, Chapman et al. 2012). This form of partial migration is commonly referred to as partial anadromy (see Chapman et al. 2012). Partial migration can also occur in non-anadromous populations, i.e., migration between distinct freshwater habitats (Jonsson and Jonsson 2011, Chapman et al. 2012). The advantage of migration include the opportunity to access better feeding areas to enhance growth and fecundity (Lucas and Baras 2001, Jonsson and Jonsson 2011, Brönmark et al. 2013). On the other hand, migration comes with costs such as increased energy expenditure and risk of predation (Lucas and Baras 2001). Furthermore, anadromous individuals that cross the interface between freshwater and seawater must undergo energy-demanding physiological changes (Brönmark et al. 2013). Especially the transition from freshwater to marine environments of juvenile salmonids is a critical life history phase that is typically characterized by high levels of mortality (Thorstad et al. 2011a, 2012a). The preparatory morphological, physiological, and behavioral changes preceding seaward migration of juvenile salmonids, a process known as smoltification, is described in Section 2.

1.3. Life cycle

Atlantic salmon and brown trout spawn in freshwater during autumn or winter. With a few rare exceptions, both species spawn in running waters, such as brooks, streams, and rivers (Elliott 1994, Jonsson and Jonsson 2011). At the spawning site, the female digs a shallow depression (also termed spawning nest) in the bottom gravel with the caudal fin. The eggs are then deposited in the spawning nest and fertilized by one or more males (Jonsson and Jonsson 2011). After spawning, the female covers the fertilized eggs with gravel and smaller stones to protect them from disturbance and predators. The female usually deposits the eggs in several spawning nests (Fleming 1996). The fertilized eggs develop over winter and the alevins hatch during the subsequent spring. The alevins remain within the spawning nest and feed endogenously on their yolk sac during the first 3 to 8 weeks, whereupon they emerge from the gravel and start exogenous feeding and defending territories as fry. As the fry continue to grow, vertical dark lines and spots (parr marks) begin appearing along their body. At this stage the juvenile fish are referred to as parr. In anadromous populations, the parr spend 1 to 9 years in freshwater before they transform into smolts and start their seaward migration (Klemetsen et al. 2003, Jonsson and Jonsson 2011, Thorstad et al. 2011a; see Section 2). Once the smolts enter the saltwater environment, and until the end of the first sea-winter, they are termed post-smolts. After 1-4 years in the marine environment mature adults return to their natal river or stream for spawning (Økland et al. 1993, Elliott 1994, Klemetsen et al. 2003). Atlantic salmon and brown trout are iteroparous, and are thus able to spawn more than once in their lifetime. In anadromous populations of both species, male parr may mature sexually in freshwater (precocious male parr) before migration to sea. Precocious male parr may spawn again as parr or anadromous adults (Klemetsen et al. 2003, Thorstad et al. 2011a).

2. SMOLT TRANSFORMATION AND MIGRATION

Anadromous Atlantic salmon and brown trout undergo a number of separated, but co-ordinated developmental changes in morphology, physiology, and behavior prior to the first seaward migration. This process has been termed parr-smolt transformation or smoltification. This section opens with a brief review of important physiological and morphological changes associated with the smoltification process and describes the typical smolt age, size, and sex ratio. Next, the timing and duration of the seaward migration as well as aspects of behavioral changes occurring during the downstream migration of smolts are synthesized. This is followed by a short discussion of some important natural and anthropogenic factors that may influence survival of smolts during the migration. The background information about the smolt transformation and migration presented in this section is used as basis for discussions in later sections of the thesis.

2.1. Smoltification

2.1.1. Smolt age, size, and sex ratio

As noted previously, Atlantic salmon and brown trout usually undergo smoltification at ages between 1 and 9 years, with population means ranging from less than 2 years to more than 4 years (Jonsson and L'Abée-Lund 1993, Økland et al. 1993, Hutchings and Jones 1998). In brown trout, smolt age generally increases with latitude as a result of lower growth potential in northern rivers (L'Abée-Lund et al. 1989, Jonsson and L'Abée-Lund 1993). This relationship is weaker in Atlantic salmon (Metcalfe and Thorpe 1990, Jonsson and Jonsson 2011). In Denmark, the average smolt age for brown trout has been found to vary between 1.8 and 2.2 years (Christensen et al. 1993), whereas the average smolt age for Atlantic salmon range from 1.5 to 1.9 years (Larsen 1950). Within the same river, the average smolt age for both species may vary across years due to fluctuations in local abiotic and/or biotic factors, such as water temperature, fish density, and food availability (Jonsson and Jonsson 2011).

The average length of Atlantic salmon and brown trout smolts is usually 100 to 200 mm in total body length (TL), with a range from 70 to 300 mm (Jensen and Johnsen 1986, Jonsson and L'Abée-Lund 1993, Jonsson et al. 1998, Jonsson and Jonsson 2011, Thorstad et al. 2012a). Hence, there exists no universal size threshold for smoltification (Økland et al. 1993, Jonsson and Jonsson 2011).

In both species, females have a higher tendency to undergo smoltification and migrate to sea than males (Christensen et al. 1993, Jonsson et al. 1998, Jonsson and Jonsson 2011). The overrepresentation of females among migrating smolts is probably because fecundity is more closely related with body size in females than males.

2.1.2. Morphological and physiological changes

For anadromous Atlantic salmon and brown trout, the decision whether to smoltify and migrate to sea in spring or delay smoltification for one or more years, or alternatively mature in freshwater, is hypothesized to be made at the end of the preceding summer and autumn in response to both local environmental conditions and individual physiological state (Metcalfe et al. 1989, Thorpe et al. 1992, 1998, Olsson et al. 2006, Boel et al. 2014). Individuals that migrate to seawater are generally characterized by maintaining high growth rates during late summer and autumn the year before migration, while slower growing individuals are more likely to postpone migration or mature in freshwater (Metcalfe et al. 1989, Metcalfe 1998, Thorstad et al. 2012a). As such, individuals with high standard metabolic rate and food requirements tend to migrate from freshwater to marine habitats (Boel et al. 2014, Kendall et al. 2015). There is also evidence suggesting that individuals with higher lipid levels in autumn have increased propensity to mature in freshwater instead of migrating to the sea (Thorstad et al. 2012a, Boel et al. 2014, Kendall et al. 2015).

During smoltification, the juvenile fish undergo a number of morphological and physiological changes in preparation for life in seawater (Hoar 1976, 1988, Jonsson and Jonsson 2011). The morphological changes include a slimmer body form (increase in length relative to mass), darkened caudal and pectoral fins, and silvering of the body sides which covers the parr marks (Wedemeyer et al. 1980, McCormick et al. 1998a, Jonsson and Jonsson 2011). Furthermore, the dorsal surface darkens and the ventral areas become brighter. These alterations in body coloration are presumably adaptive for predator avoidance in the pelagic environment. Development of hypo-osmoregulatory ability is among the most important processes during smoltification (Hoar 1988, Boeuf 1994, McCormick et al. 1998a, Thorstad et al. 2012a). This physiological change is associated with increased gill and intestinal Na^+,K^+-ATPase activity and differentiation of chloride cells, resulting in greater salt secretory capacity and increased salinity tolerance. The physiological changes also include increases in circulating levels of thyroid hormones, cortisol, growth hormone, and insulin-like growth factor I (Hoar 1988, Boeuf 1994, Dickhoff et al. 1997, McCormick et al. 1998a, Jonsson and Jonsson 2011). These hormones stimulate the development of seawater tolerance through chloride cell proliferation and differentiation in the gills (Jonsson and Jonsson 2011). The initiation of the physiological smolt transformation is cued by photoperiod (increased day length) and to a lesser extent water temperature (Wedemeyer et al. 1980, McCormick et al. 1998a, Jonsson and Jonsson 2011). Water temperature seems to control the rate of smoltification (McCormick et al. 1998a).

2.2. Smolt migration

2.2.1. Timing and duration of smolt migration

Once the juvenile fish are morphologically and physiologically prepared for saltwater entry, environmental cues are often required to onset the smolt migration (McCormick et al. 1998a, Riley et al. 2002, Jonsson and Jonsson 2011). Water temperature and discharge are generally considered the main environmental factors triggering the downstream migration (Jonsson and Ruud-Hansen 1985, Bohlin et al. 1993, 1996, Aldvén et al. 2015). It has been shown that, in some rivers, the smolt migration takes place as water temperature reaches a certain threshold temperature (Jonsson and Ruud-Hansen 1985). However, there appear to be no universal threshold temperature initiating the smolt migration (Jonsson and Ruud-Hansen 1985, Hvidsten et al. 1995, Jonsson and Jonsson 2011). For example, in some rivers the smolt migration begins at water temperatures as low as 2 to 4 ºC (Hvidsten et al. 1995, Jonsson and Jonsson 2011), while in other rivers, the downstream migration starts at water temperatures between 5 to 11 ºC (Jonsson and Ruud-Hansen 1985). Other researchers have suggested that cumulative temperature (i.e., degree-days) experienced by the smolts is a better indicator of the initiation of smolt migration than is absolute temperature (Zydlewski et al. 2005). As a result, the timing of smolt migration usually depends upon latitude, with smolts from northern rivers migrating to sea later than those from more southerly rivers (Hvidsten et al. 1998, Thorstad et al. 2011a, 2012a). The environmental cues may be of varying importance in initiating and controlling smolt migration among different populations, probably reflecting different local adaptations to ensure high survival at sea entry (Hvidsten et al. 1998, McCormick et al. 1998a, Thorstad et al. 2011a). The relative importance of water temperature and discharge in initiating the smolt migration may also vary annually within the same river system (Hembre et al. 2001, Aldvén et al. 2015).

The smolt migration of Atlantic salmon and brown trout typically occurs in spring and early summer (McCormick et al. 1998a, Jonsson and Jonsson 2011, Thorstad et al. 2011a, 2012a). In Denmark, the seaward migration of brown trout smolts usually takes place from March to early June (Koed 1995, Thomassen 1998, Aarestrup et al. 2002; **paper I**), while Atlantic salmon smolts migrate downstream from early April to end of May (Rasmussen 1986, Koed 1995). The smolt migration in Danish rivers and streams generally extends over a 3 to 7 week period with the majority of individuals in a population migrating within a 1 to 2 week period (Aarestrup et al. 2000; **paper I**). Typically, the peak migration of Atlantic salmon and brown trout smolts occurs from mid-April to early May in Danish rivers and streams (Aarestrup et al. 2000, 2002).

The size and age of Atlantic salmon and brown trout smolts have been shown to affect the seasonal timing of the seaward migration. In general, larger and older smolts migrate earlier in the season compared to smaller and younger conspecifics (Rasmussen 1986, Bohlin et al. 1993, 1996). In addition, the timing of smolt migration may be genetically determined (Aarestrup et al. 1999, Stewart et al. 2006, Jonsson and Jonsson 2011).

2.2.2. Smolt window and desmoltification

The timing of the smolt migration is crucial for survival in marine environments, and it is believed that smolts utilize environmental cues in the river that are synchronized with favorable temperature and food conditions at sea (Pickering 1989, Hvidsten et al. 1998, 2009, Byrne et al. 2004, Thorstad et al. 2012a). This is known as the ecological smolt window, where the environmental conditions at sea are suitable for high smolt survival (McCormick et al. 1998a). The physiological readiness of smolt for migration and seawater entry is another important aspect influencing survival (McCormick et al. 1998a). There is only a limited period of time during which smolts are at peak physiological preparedness for seaward migration, known as the physiological smolt window (McCormick et al. 1998a, 2009). If smolts remain in freshwater past the ecological and physiological smolt windows they lose their silvery color and exhibit reduced hypo-osmoregulatory ability (McCormick et al. 1998a, 1999, Stefansson et al. 1998, Jonsson and Jonsson 2011). This process is known as desmoltification. Delays of smolt migrations caused by anthropogenic barriers, such as dams and weirs, may therefore result in desmoltification and increased mortality (Aarestrup and Koed 2003, Garcia de Leaniz 2008, Marschall et al. 2011, Thorstad et al. 2012a).

2.2.3. Migration behavior

During the seaward migration, the smolts display decreased agonistic and territorial behavior (Jonsson and Jonsson 2011, Thorstad et al. 2012a). They often form shoals during migration (Bakshtanskiy et al. 1988, Hvidsten et al. 1995, Jonsson and Jonsson 2011), a strategy which is thought to reduce individual risk of predation (Jonsson and Jonsson 2011). The smolt migration usually takes place during night, but as water temperature rises towards the end of the migration period an increasing number of smolts migrate during daytime (Ibbotson et al.

2006, Koed et al. 2006, Jonsson and Jonsson 2011). Nocturnal migration is believed to be another predator avoidance tactic against visual predators. At high latitude areas with midnight sun, the riverine migration may occur during both day and night (Bakshtanskiy et al. 1980, Veselov et al. 1998, Carlsen et al. 2004, Davidsen et al. 2005). Increased water discharge and turbidity can also increase the proportion of smolts migrating during daytime (Solomon 1978).

The smolts often migrate downstream in near surface waters in the middle part of the river channel with the highest water velocities (Moore et al. 1998, Davidsen et al. 2005). However, migration in the deepest part of the water column has also been recorded (Davidsen et al. 2005, Svendsen et al. 2007). The riverine migration was first believed to be a passive displacement with the current (Thorpe and Morgan 1978, Tytler et al. 1978, Thorpe et al. 1981), but several studies have now demonstrated that the movement of smolts may also be active (Fängstam 1993, Svendsen et al. 2007). The net ground speed during downstream migration has been shown to vary considerably both among Atlantic salmon and brown trout smolts. Studies on Atlantic salmon have reported riverine migration speeds from 0.2 to 28 km day^{-1} (Ruggles 1980, Aarestrup et al. 2002). Individual net ground speed of brown trout smolts during seaward migration may vary from 1 to 60 km day^{-1} within the same population (Aarestrup et al. 2002). The variation in smolt migration speed among populations may be related to differences in local environmental conditions (Aarestrup et al. 2002). For example, it has been showed that Atlantic salmon and brown trout smolts migrated more quickly during high than low water flows (Aarestrup et al. 2002). In addition, migration speed of smolts tends to be slower in smaller streams than in larger rivers (Ruggles 1980, Aarestrup et al. 2014).

2.2.4. Autumn and winter migration

Autumn downriver migration of Atlantic salmon and brown trout pre-smolts has been reported from rivers on both sides of the North Atlantic Ocean (Pemberton 1976, Riddell and Leggett 1981, Cunjak et al. 1989, Poole et al. 1996, Jonsson and Jonsson 2002, Ibbotson et al. 2013). These fish seem not to be fully morphologically and physiologically adapted to seawater conditions (Poole et al. 1996, Riley et al. 2008), and it is presently unknown whether they overwinter in freshwater or low saline areas in the lower reaches of the river instead of migrating to sea (Thorstad et al. 2011a). Jonsson and Jonsson (2002), for example, observed downstream migration of juvenile brown trout from September to January in a Norwegian river and suggested that they overwintered in brackish water in or near the river mouth. However, it remains unknown if these trout migrated further offshore (Jonsson and Jonsson 2002). In **paper II,** we also found evidence for winter migration of juvenile brown trout by use of passive integrated transponder (PIT) telemetry in Gudsø Stream, located in eastern Jutland, Denmark (Fig. 1). From 7 November 2012 to 28 February 2013, 49% of all migratory control fish passed a downstream PIT antenna station (S1; see Fig. 1). An important caveat to note regarding this observation is that there is still approximately 1 km of stream habitat downstream of the antenna station before the stream enters Kolding Fjord (Fig. 1). Because electrofishing was not conducted from S1 to the stream outlet into the fjord during or after the study, it cannot be conclusively stated whether these individuals migrated to sea during winter or became resident in the lower reaches of the stream. It is also plausible that the trout completed smoltification in this stream section and entered the fjord the subsequent spring. Since none of these juvenile trout were detected moving back upstream, it is unlikely that they used the lower stream section solely as overwintering habitat, unless they all died before a potential upstream migration event. Positioning of detection antennae closer to the fjord or analysis of return data of adult sea trout could help resolve the ultimate destination of these types of individuals. Finally, it should be stressed that the absolute number of winter migratory brown trout was low in our study (n = 23). Additional studies are required to investigate the incidence as well as environmental and physiological drivers behind autumn and winter migrations of juvenile salmonids. These studies should also attempt to resolve if the occurrence of autumn and winter migrations are linked to fish age, sex, and size. Moreover, it is of general and applied interest to determine whether and to what degree these individuals enter seawater environments during autumn or winter.

2.3. Mortality during smolt migration

Predation by fish, birds, and mammals is often a major source of natural mortality of migrating Atlantic salmon and brown trout smolts in streams and rivers (Ward and Hvidsten 2011, Thorstad et al. 2012a). These predatory species include; pike (*Esox lucius*), pikeperch (*Sander lucioperca*), burbot (*Lota lota*), brown trout, grey heron (*Ardea cinerea*), cormorant (*Phalacrocorax carbo*), gulls (*Larus* spp.), American mink (*Neovison vison*), and European otter (*Lutra lutra*) (Larsson 1985, Heggenes and Borgstrøm 1988, Jepsen et al. 1998, 2000, Aarestrup et al. 1999, Koed et al. 2002, 2006, Jacobsen 2005, Boel 2012). Estimates of predator-induced mortality during smolt migration have been found to vary considerably among rivers (Thorstad et al. 2012a). For instance, in a recent review Thorstad et al. (2012a) summarized existing studies and reported natural mortalities ranging from 0.3 to 7.0% km^{-1} during the downstream migration of Atlantic salmon smolts.

In many catchments, smolts have to swim through lakes or reservoirs along the downstream migration route to the sea (Jepsen et al. 1998, Aarestrup et al. 1999, Koed et al. 2002). Mortality of smolts is often particularly high in these systems as lakes and reservoirs provide favorable habitats for many predatory fish and bird species (Jepsen et al. 1998, 2000, Aarestrup et al. 1999, Koed et al. 2002, 2006). Jepsen et al. (1998) reported 90% mortality of Atlantic salmon and brown trout smolts in a shallow man-made reservoir due to predation by pike and birds. In **paper I**, we found a loss of ~15% wild brown trout smolts in a small millpond (~0.2 ha) located in Gudsø Stream by use of PIT telemetry (i.e., PIT antenna arrays were installed upstream and downstream of the millpond; see Fig. 1). Mortality was probably mainly due to predation by grey heron and cormorants, which were frequently observed foraging in the millpond during the study.

Power stations, dams, and weirs can also increase mortality of smolts during the downstream migration (Ruggles 1980, Aarestrup and Koed 2003, Johnsen et al. 2011, Thorstad et al. 2012a). For instance, Aarestrup and Koed (2003) showed smolt losses between 18 and 71% for Atlantic salmon and brown trout due to passage of weirs (0.6–2.5 m high) associated with fish farms in lowland streams. The authors attributed these smolt losses to individuals penetrating grids erected at the fish farm inlets, increased predation at the intake grid, and migration delays that may have resulted in desmoltification (Aarestrup and Koed 2003). Mortality of Atlantic salmon smolts related to power station turbine passage has been reported to vary from 7 to 80% (reviewed by Johnsen et al. 2011). Smolts may be killed or severely injured by rotating parts of the turbine or extreme turbulence associated with water intake and outflow (Johnsen et al. 2011, Thorstad et al. 2012a). It should be noted that power stations, dams, weirs, and other man-made barriers may also disturb the upstream spawning migration of adult salmonids and even prevent fish from reaching the spawning grounds (Aarestrup and Jepsen 1998, Thorstad et al. 2003, 2008). This has led to extirpation or declines of several anadromous populations of Atlantic salmon and brown trout (Parrish et al. 1998, Rasmussen and Geertz-Hansen 2001, Johnsen et al. 2011; see Section 4).

Contaminants and organic pollutants can have significant impact on smolt development, migration behavior, and ultimately survival (McCormick et al. 1998a, 2009, Moore et al. 2003, 2007, Rosseland and Kroglund 2011, Thorstad et al. 2012a). For instance, Moore et al. (2007) showed that exposure to an agricultural pesticide (atrazine) inhibited migratory activity and modified olfactory sensitivity of Atlantic salmon smolts. Hence, it is believed that pesticides can have implications for olfactory imprinting of the natal river and subsequent homing migration of mature adults (Moore et al. 2007, Thorstad et al. 2012a). Several studies have shown that acidification and the concurrent mobilizing of aluminum (Al) can severely damage fish gills and influence smolt development and saltwater tolerance (reviewed by Rosseland and Kroglund 2011). In fact, acidification has been the main responsible factor for extinction or severe reductions of Atlantic salmon populations in several Norwegian rivers (Sandøy and Langåker 2001).

To summarize, the downstream migration route to the sea often poses a wide variety of challenges that can reduce the probability of successful migration (McCormick et al. 2009). Predation is perhaps one of the most obvious factors. However, environmental challenges that do not immediately kill fish can initiate a long array of stress responses that may have later implications for an individual’s fitness (e.g., growth and survival) and seaward migration success (McCormick et al. 1998a, 2009, Björnsson and Bradley 2007). In the next section, I discuss the possible consequences of prolonged stress on fitness and downstream migration of juvenile salmonids.

3. EFFECTS OF STRESS ON SMOLT MIGRATION

The overall goal of this section is to provide insight into the potential impact of stress on the downstream migration success of juvenile salmonids. It begins with a description of the physiological stress response in fish. This is followed by a brief introduction to the ecology of stress to give a few examples of natural and anthropogenic stressors that have the potential to influence individual performance and whole population-level processes. Finally, the main results of two complementary studies (referred to as **paper I** and **II**) that aimed to investigate the effects of prolonged elevation of plasma cortisol levels on growth, survival, and seaward migration success of juvenile brown trout are summarized and discussed. Before continuing, it is important to recall that cortisol plays a pivotal role in the parr-smolt transformation and it is well known that plasma cortisol concentrations increase during this period (e.g., Wedemeyer et al. 1980, Jonsson and Jonson 2011). Cortisol stimulates differentiation of chloride cells and increases gill Na^+,K^+-ATPase activity, resulting in greater seawater tolerance (Jonsson and Jonsson 2011; see Section 2). However, cortisol is also released when fish are stressed (see below). Therefore, smoltification is a highly relevant life-history phase to examine effects of stress on fitness-oriented endpoints such as growth, survival, and seaward migration.

3.1. Stress response in fish

The concept of stress has been debated for several decades and there is as yet no complete agreement among scientists with respect to the correct terminology (see Barton 1997). The difficulties associated with defining stress can be illustrated by the following quotation by Levine (1985): *"I am not certain whether one who undertakes this task (of defining the concept of stress) either has an enormous ego, is unmeasurably stupid, or is totally mad"*. Many experts favor the original definition proposed by Hans Selye in 1936, who defined stress as *"the non-specific response of the body to any demand made on it"*. However, several modifications to Selye's original definition have been suggested. For instance, Brett (1958) defined stress as *"a state produced by an environmental or other factor which extends the adaptive responses of an animal beyond the normal range or which disturbs the normal function to such an extent that, in either case, the chances of survival are significantly reduced"*. Other researchers have defined stress as any threat to or disturbance of homeostasis, implying that stress not necessarily has detrimental effects on organismal performance (Hinkle 1987, Barton and Iwama 1991). In order to keep things as simple as possible, the definition of stress used in this thesis is a stimulus perceived by the body that elicits a stress response (Romero 2004).

Existing literature has categorized the stress response of fish into three different stages: primary, secondary, and tertiary responses (reviewed in Barton 2002). The first step of the primary stress response involves a rapid release of catecholamines (e.g., adrenaline and noradrenaline) into the blood stream from the chromaffin tissue located in the head kidney (Sumpter 1997, Mommsen et al. 1999, Barton 2002). This is followed by activation of the hypothalamus-pituitary-interrenal (HPI) axis, resulting in release of corticosteroid hormones into the blood (Sumpter 1997, Barton 2002). In brief, the pathway for release of corticosteroids begins with secretion of corticotropin-releasing hormone (CRH) by the hypothalamus in the brain. CRH triggers the pituitary gland in the brain to release adrenocorticotropin (ACTH) which in turn stimulates the interrenal tissue embedded in the head kidney to synthesize and release corticosteroids, primarily cortisol (Sumpter 1997, Barton 2002). The synthesis and release of cortisol takes a few minutes and its effects can last for hours or days, depending on the intensity and duration of the stressor (Mommsen et al. 1999, Sapolsky et al. 2000). The release of stress hormones into circulation evokes the secondary response, which may include increases in ventilation rate, oxygen uptake, heart rate, and blood pressure, as well as mobilization of energy (e.g., depletion of liver and muscle glycogen) and disturbances in osmoregulation and ion balance (Mazeaud et al. 1977, Barton 1997, 2002, Sumpter 1997). Specifically, elevated levels of catecholamines facilitate energy to fight or flee from a threat, whereas cortisol plays an important role in mobilizing energy that allows the fish to preserve its normal homeostatic state (Sapolsky et al. 2000, Barton 2002). Hence, the stress response is initially considered an adaptive mechanism that serves to increase the likelihood of the individual surviving and recovering from a stress event (Barton 2002). However, the physiological stress response mechanism may become maladaptive for whole-body activity and performance if the intensity of the stressor is severe or long-lasting (see reviews by Barton and Iwama 1991, Wendelaar Bonga 1997, Barton 2002). These tertiary responses include reductions in condition, metabolic scope for growth, immune function, reproductive output, and ultimately survival (Barton 1997, 2002, Schreck 2010). Circulating plasma cortisol titers are commonly used as a physiological indicator of stress levels in fish due to the central role of cortisol in the stress response mechanism (Barton 2002).

3.2. Ecology of stress

Wild populations of fish are exposed to a broad scope of both natural and anthropogenic stressors. Natural stressors include predation attempts, food scarcity, inter- and intraspecific interactions, disease, parasitism, and reduced oxygen concentration as well as rapid changes in water discharge and temperature (Strange and Schreck 1978, Fraser and Gilliam 1992, Wedemeyer 1997, Flodmark et al. 2002, Gilmour et al. 2005). Potential sources of anthropogenic stressors include habitat alterations (e.g., dams, weirs, locks, hydropower stations, urbanization, and channel modifications), pollution (e.g., pesticides, organic matter, and heavy metals), boat noise, and angling (Wang et al. 2000, Rasmussen and Geertz-Hansen

2001, Arlinghaus et al. 2007, McCormick et al. 2009, Thorstad et al. 2012a, Jacobsen et al. 2014; see Section 2). Both natural and anthropogenic stressors are highly variable on a temporal scale. For instance, avoiding a predator may cause only transient plasma cortisol elevations, whereas exposure to habitat alteration or pollution may cause elevated cortisol levels over an extended period of time. When brown trout are exposed to acute stressors, plasma cortisol typically rises to between 100-150 ng ml^{-1} within 1-2 hours (Pickering et al. 1982). Plasma cortisol concentrations gradually decline back to baseline levels (<5 ng ml^{-1}) after 24-48 hours (Pickering et al. 1982). By comparison, a chronic stressor may elevate plasma cortisol for several weeks, but at lower magnitude (Pickering and Stewart 1984). In general, the cortisol levels of chronically stressed brown trout range from 10 to 20 ng ml^{-1} (Pickering and Stewart 1984, Pickering and Pottinger 1987). It should be noted that there is considerable variability in the magnitude and duration of the cortisol response among fish species (reviewed in Pankhurst 2011). Even individuals in the same population may exhibit significant variation in post-stress plasma cortisol levels (e.g., Koolhaas et al. 2010).

Although most stressors do not immediately kill fish, they may have long-lasting effects on individual performance. For example, largemouth bass (*Micropterus salmoides*) exposed to an early spring stressor of relatively short duration (6 days) exhibited reduced growth rates during summer (O’Connor et al. 2011). This phenomenon is known as carryover effects, whereby the current state of an individual is influenced by its previous experience (Norris 2005, Harrison et al. 2011, O’Connor et al. 2014). More specifically, O’Connor et al. (2014) define carryover effects as: ”*In an ecological context, carryover effects occur in any situation in which an individual’s previous history and experience explains their current performance in a given situation*”. Carryover effects of stress that occur on the individual level may also have significant repercussions for the long-term structure and viability of their population (O’Connor et al. 2011, 2014). It is presently not known how common and important these effects are in nature (Harrison et al. 2011, Pankhurst 2011).

Most of our present knowledge with respect to how fish respond to stress has been obtained from cultured or wild fish acclimated to laboratory conditions (reviewed by Barton 2002, Pankhurst 2011). Moreover, results from laboratory studies have often been extrapolated to predict ecological consequences of stress in wild fish populations (Pankhurst 2011). This pattern may be attributed to the technical difficulties associated with studying the impact of stressors on fish in the wild (e.g., controlling for environmental history). To fully understand the effects of stress on wild fish populations, it is essential that studies are conducted in nature, where environmental complexity is higher and stressors are more dynamic. Exogenous manipulation of plasma cortisol titers has increasingly been recognized as a promising technique to mimic the physiological effects of exposure to a stressor in fish. Cortisol implants may therefore be utilized to expand our understanding of stress ecology in wild fish (see next subsection).

3.3. Use of hormone implants to manipulate cortisol titers

Intra-coelomic injection of cortisol dissolved in cocoa butter is an increasingly used method for artificially increasing circulating levels of plasma cortisol in teleost fishes (reviewed in Gamperl et al. 1994). Implants are prepared by suspending a known concentration of hydrocortisone 21-hemisuccinate in liquid cocoa butter at 40 ºC (melting temperature of cocoa butter). Following injection, the cocoa butter rapidly solidifies within the peritoneal cavity of the fish. Cocoa butter implants are particularly useful as they have been shown to deliver corticosteroids at a relatively stable rate over an extended period rather than causing a transient increase in plasma cortisol levels with saline or coconut oil injections (Pickering 1989, Gamperl et al. 1994). In addition, fish likely recover from the initial stress associated with the injection and handling before the effects of the releasing hormones are lost from the cocoa butter implant (Gamperl et al. 1994). For example, Pickering (1989) found that a dosage of 30 mg cortisol per kg body mass raised circulating plasma levels to 15-20 ng ml^{-1} for approximately 3 weeks in juvenile brown trout. This level of plasma cortisol elevation is well within the physiological range for chronically stressed brown trout (see above; Pickering and Stewart 1984, Pickering and Pottinger 1987, Pickering 1989). At the same time, circulating cortisol levels of sham-implanted (i.e., injection of plain cocoa butter) brown trout usually remain below 4 ng ml^{-1} (Pickering and Duston 1983, Pickering 1989). This plasma cortisol level corresponds well to typical baseline values for brown trout (see above; Pickering et al. 1982). Post-injection cortisol concentrations in the blood stream of brown trout are dose dependent and positively correlated with water temperature (Pickering 1989, Gamperl et al. 1994).

Experimental cortisol elevations are associated with the same secondary and tertiary responses as natural and anthropogenic stressors (Gamperl et al. 1994). However, it is important to note that exogenous cortisol manipulations do not mimic the sensory aspects of the neuro-endocrine activation of the HPI axis associated with the stress response mechanisms (Gamperl et al. 1994). Previous studies on salmonids have shown that experimental cortisol elevations can elicit changes in the following ways: increase standard metabolic rate as well as reduce feeding motivation and growth in rainbow trout (*Oncorhynchus mykiss*) (Morgan and Iwama 1996, Gregory and Wood 1999), reduce social status and competitive ability in rainbow trout (DiBattista et al. 2005), impair innate immune response in Atlantic salmon parr (Gadan et al. 2012), and increase susceptibility of coho salmon (*Oncorhynchus kisutch*) to salmon lice (*Lepeoptheirus salmonis*) infections (Johnson and Albright 1992). Several hundred studies have used cortisol injections to assess how fish respond to natural and anthropogenic stressors, but remarkably few have been conducted in natural settings (but see O'Connor et al. 2010, 2011, Nagrodski et al. 2013). While laboratory studies provide controlled conditions, they often fail to capture the complexity of natural systems. Hence, an important objective of this thesis was to elucidate how wild fish respond to stress in their natural environment. Specifically, we experimentally manipulated cortisol titers by use of cocoa butter implants to assess the impact of a chronic stressor on growth, survival, and seaward migration success of juvenile brown trout in a small natural stream. This resulted in two separate studies. In the next subsection, I briefly describe the rationale and predictions of these experiments and summarize the main results.

3.4. Effects of cortisol manipulation on fitness and seaward migration of brown trout

The parr-smolt transformation is an inherently physiologically demanding process (Hoar 1988, McCormick et al. 2009; Section 2). Furthermore, as noted previously, the seaward migration is associated with increased energetic costs and smolts are often exposed to high levels of predation along the downstream migration route (see Section 2). These circumstances make the smolt life stage particularly vulnerable to external perturbations (Barton et al. 1985, McCormick et al. 1998a, 2009, Björnsson et al. 2011). There are several potential pathways through which stress may affect the parr and smolt stages:

- *Growth and smolt status*. Previous studies have documented that prolonged stress can lead to depletion of lipid resources due to increased metabolic rate (Sheridan 1986, Barton 1997, Wendelaar Bonga 1997, Mommsen et al. 1999). Elevation of blood plasma cortisol levels may thus reduce condition and growth of salmonids (Barton et al. 1987, McCormick et al. 1998b, Jentoft et al. 2005). Since growth trajectories are functionally linked to smoltification, stress-induced impairment of growth may indirectly determine smolt status (i.e., whether parr will smoltify or become resident).

- *Mortality*. It is known that chronic cortisol elevations can result in mortality of fish (McDonald and Milligan 1997, Barton 2002). Increased stress-mediated mortality of parr and smolts can potentially have severe ramifications for the long-term structure and dynamics of local salmonid populations.
- *Behavior*. Stress has been shown to alter fish behavior in several different ways (reviewed by Schreck et al. 1997). Stress-induced changes in behavior of smolts likely have the potential to increase risk of predation during the downstream migration (Olla et al. 1992, 1995, Mesa 1994). These behavioral changes could include elevated locomotor activity, reduced shelter seeking, and impaired predator avoidance ability (Mesa 1994, Schreck et al. 1997). Alternations in habitat use, diurnal migration pattern, and net ground speed during seaward migration due to a stressor can also be hypothesized to influence susceptibility to predation (Sigismondi and Weber 1988, Schreck et al. 1997, Ibbotson et al. 2006).
- *Feeding and competitive ability*. Increased levels of circulating cortisol can negatively influence feeding motivation (appetite) and competitive ability of salmonids (Schreck et al. 1997, Gregory and Wood 1999, DiBattista et al. 2005). Outcomes could be reduced growth and survival.
- *Immune system*. In general, chronic or repeated stress has suppressive effects on the immune system in fish (reviewed by Barton 1997, Wendelaar Bonga 1997, Weyts et al. 1999). The potential outcomes are reduced growth and/or increased mortality due to fungal and bacterial pathogens (Pickering and Pottinger 1987, Ashley 2007). Prolonged cortisol elevation has also been shown to increase susceptibility to parasites (Johnson and Albright 1992), which in turn may reduce the likelihood of successful migration to the sea or survival in marine habitats.

Thus, it appears that stress has large potential to affect fitness (e.g., growth and survival) and seaward migration success of juvenile salmonids. As mentioned, this thesis includes two distinct studies that use exogenous cortisol administration in combination with PIT telemetry to examine the effects of chronic stress on survival, growth, and seaward migration success of wild brown trout. These studies are referred to as **paper I** and **II**. Both studies were conducted in Gudsø Stream (Fig. 1). Including a series of tributaries, the stream flows approximately 16 km through primarily agricultural areas before reaching the sea at Kolding Fjord. The width of the stream is mostly between 1 to 4 m. The bottom substratum consists mainly of coarse sand, mud, and gravel. There is an old mill with a dam and millpond located approximately 1 km upstream of Kolding Fjord. PIT antenna stations covering the entire stream width were installed upstream (S1) and downstream (S2) of the millpond (Fig.1). The brown trout population in the stream exhibit partial anadromy.

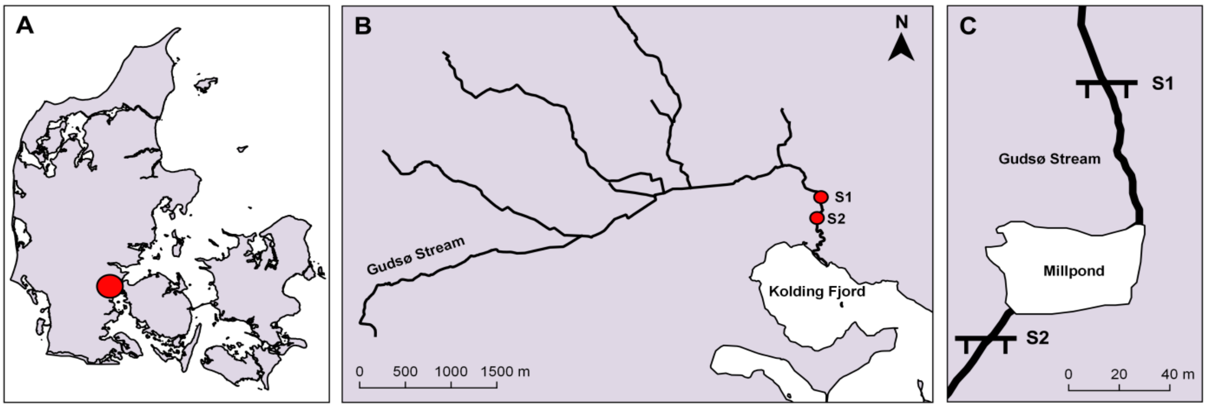

Figure 1. (A) Inserted red circle indicates the location of Gudsø Stream. (B) Gudsø Stream and the location of the PIT antennae stations (S1 and S2). (C) A millpond is situated between S1 and S2 and serves as a potential sink for migrating salmonids.

In **paper I**, we manipulated plasma cortisol titers in early spring, immediately prior to the typical smolt migration period for brown trout in Danish lowland streams (see Section 2). We tested the null hypothesis that there is no difference in growth and migration success among 4 treatment categories: control (CO), sham (SH; plain cocoa butter), low- (LW; 25 mg kg^{-1} fish body mass) and high-dose (HI; 100 mg kg^{-1} fish body mass) cortisol. The LW treatment was designed to simulate a chronic stressor, raising plasma cortisol titers to 15-20 ng ml^{-1} for 2-4 weeks (Pickering 1989). The HI treatment was designed to elicit a more extreme cortisol response that still fall within the physiological ranges for brown trout (i.e., 20-40 mg ml^{-1}; Pickering and Stewart 1984, Pickering and Pottinger 1987, Pickering 1989). Results showed that passage at S2 was significantly lower for HI brown trout when compared to the CO and SH groups (Fig. 2A).

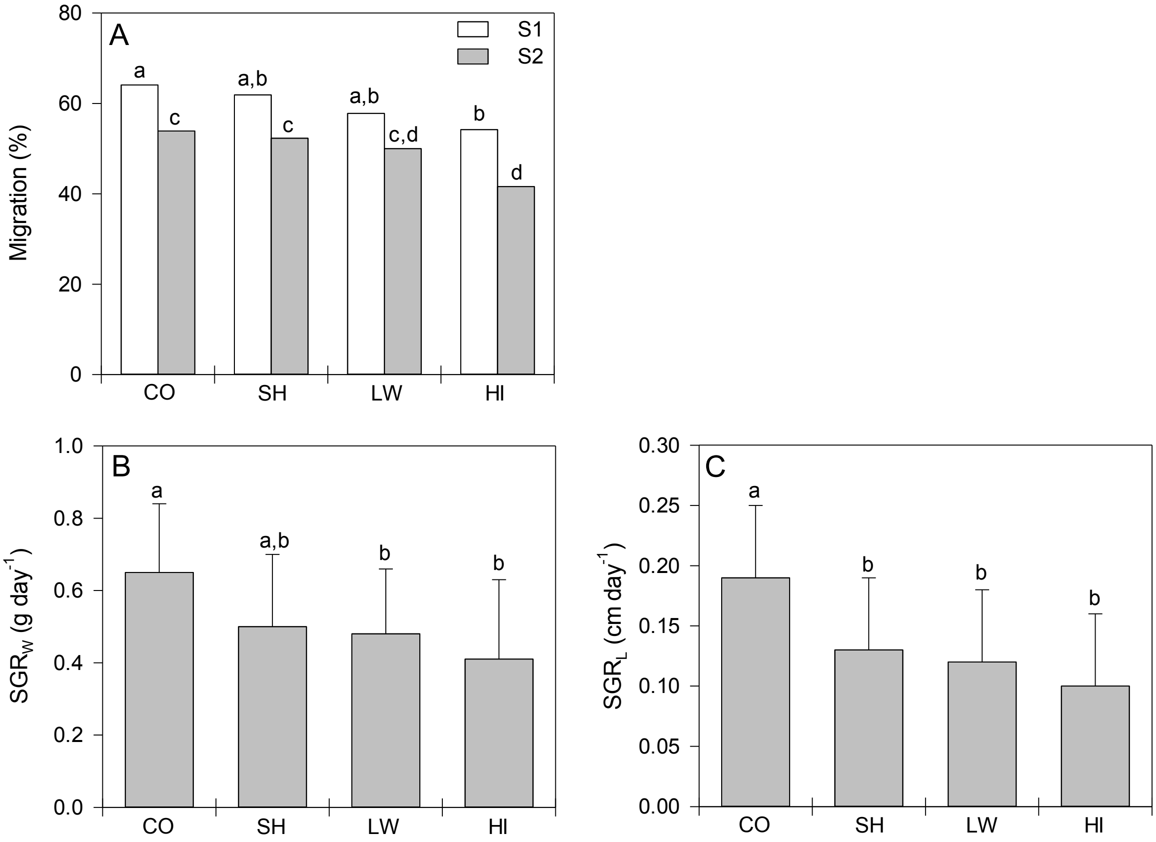

Figure 2. (A) Migration success (%) to PIT antennae stations (S1 and S2) in Gudsø Stream of brown trout (*Salmo trutta*) in the 4 treatment groups: CO (control), SH (injected with plain cocoa butter), LW (treated with 25 mg kg^{-1} solution of cortisol), and HI (treated with 100 mg kg^{-1} solution of cortisol). Specific growth rate for (B) mass (SGR_W; g day^{-1}) and (C) length (SGR_L; cm day^{-1}) of brown trout in the 4 treatment groups that were recaptured in the stream after the smolt migration had finished. Specific growth rate was calculated as: $SGR = (\log_e Y_f - \log_e Y_i) \times t^{-1}$, where Y_i is the mass or length at the time of tagging, Y_f is the mass or length at the time of recapture, and t is the time in days. Values are mean ± SD. Different letters denote significant differences at $p < 0.05$.

Furthermore, significantly fewer HI fish were also detected at S1 in comparison to CO individuals. There was no difference in the number of recaptures among treatment groups during electrofishing surveys that were conducted after the smolt migration had finished. Therefore, the observed decrease in passage of HI fish was likely the result of increased mortality during seaward migration. The available data do not identify the ultimate mechanism that underlies this observation. However, increased predation of HI individuals due to stress-induced changes in behavior (e.g., increased locomotor activity) before or during the downriver migration could provide a plausible explanation. Since the number of in-stream recaptures was similar between treatment group after the smolt migration, it does not appear that an early spring stressor influence smolt status. Similarly, the timing of migration did not differ among treatment groups. Growth of cortisol-treated brown trout that remained in the stream was reduced in comparison to individuals in the CO group (Fig. 2B,C). Unfortunately, it was not possible to assess growth of brown trout that migrated out of the stream. The reduced growth rate of cortisol-treated individuals that remained in the stream was likely the result of elevated metabolic rate due to the artificial spring stressor. Thus, even if fish are able to recover from a stressor, their long-term fitness may still be impaired. Such carryover effects reveal the cryptic manner in which natural and anthropogenic stressors can influence fish populations.

Winter can be an extremely challenging period for stream-dwelling brown trout due to low temperature conditions and often reduced prey availability of aquatic and terrestrial invertebrates (French et al. 2014). As a consequence, salmonids usually experience reduced growth, condition, and survival over the winter (Quinn and Peterson 1996, Biro et al. 2004, French et al. 2014). We therefore predicted that exposure to a chronic cortisol elevation in late fall could further decrease growth and the likelihood of survival in the stream during winter. This hypothesis was tested in **paper II** by artificially manipulating cortisol titers of juvenile brown trout via intercoelomic injection of cortisol (100 mg kg^{-1} fish body mass) in late fall. Hence, the magnitude of cortisol elevation targeted in **paper II** was within physiologically relevant values (Pickering and Stewart 1984, Pickering and Pottinger 1987, Pickering 1989; see above).Their overwinter and spring survival, growth, and seaward migration success the following spring were compared with a control group. The overwinter mortality was high for both the control (64%) and cortisol-treated (86%) brown trout, emphasizing that winter is a challenging time for juvenile salmonids (Biro et al. 2004, French et al. 2014). However, mortality was 2.5 times higher for cortisol-treated fish. In addition, less than half as many artificially stressed individuals were detected at the PIT antennae stations (S1 and S2) over the winter and during spring migration compared to the control treatment. Finally, while cortisol-treated brown trout exhibited negative growth of mass over the winter, control individuals continued to grow. Elevated depletion of lipid reserves due to stress-induced elevation of metabolic rate provides a likely mechanism for the lower overwinter growth and survival of cortisol-treated brown trout (see Biro et al. 2004). Increased predation of the artificially stressed fish could further have reduced survival (Olla et al. 1992, 1995). Hence, the results of **paper II** suggest that stressors have the potential to carry over across seasons and reduce growth and survival of brown trout. We caution, however, that since we did not assess the timing of mortality in cortisol-treated trout, it is possible that they experienced increased mortality shortly after treatment. Regardless, the stress-induced decrease in growth and survival may have consequences for the number of out-migrating smolts and may dedicate the future success of individuals that survive and enter the sea.

The present studies have some limitations that should be acknowledged. First, blood plasma cortisol titers in the experimental trout were not measured following injection of cortisol. Although the dose of the implant is directly proportional with the cortisol titers appearing in the blood stream of brown trout, this relationship is influenced by ambient water temperature (Pickering 1989, Gamperl et al. 1994). Thus, measurements of post-injection plasma cortisol titers from a sub-group of brown trout would have allowed us to validate whether the applied doses of cortisol resulted in physiological relevant plasma cortisol levels under the given water temperature conditions. Second, results from **paper I** indicate that the cocoa butter vector *per se* negatively affected growth of brown trout as the SH group had lower specific growth rate for length than the CO group (Fig. 2C). Furthermore, specific growth rate for mass and length did not differ between the SH group and the cortisol-treated groups (LW and HI; Fig. 2B,C). This suggests that the cocoa butter implant act as a stressor, regardless of whether the implant contains cortisol or not. That being said, there was still a decreasing trend for growth based on the dose of cortisol that was applied (Fig. 2B,C). Moreover, survival of SH fish to the PIT antenna stations was similar to that of CO individuals (Fig. 2A). We therefore believe that our results are still interesting and valid in their direction and overall conclusion. Unfortunately, sham-treated individuals were not included in **paper II** due to low numbers of captured brown trout in the desired size range (≥120 mm TL) during the surveys in the stream. Without a sham-treated group in **paper II**, we cannot be certain of the portions of change in growth that can be attributed to the cortisol treatment or the cocoa butter vector. It is apparent that follow-up empirical studies are required to validate the applicability of cocoa butter as a carrier for cortisol. As discussed by Hoogenboom et al. (2011), the observed negative impact of the plain cocoa butter on growth may be a result of immune response to the presence of foreign matter in the body cavity. It is also possible that increased energy expenditure associated with carrying the implant led to reduced growth. Tissue encapsulation of the implants could further restrict the amount of energy availably for growth (see Hoogenboom et al. 2011). Dissection of sham-treated fish would help to determine whether tissue encapsulation represent a responsible mechanism for the observed growth suppression.

Despite the limitations mentioned above, the present studies provide a first step in elucidation the nature of long-term consequences of stress in wild populations of salmonids. Collectively, these studies highlight the potential of stressors to carryover across temporal scales and different life stages. We hope that our studies will inspire other researchers to revisit the potential impacts of natural and anthropogenic stressors on juvenile salmonids as they transit from their natal freshwater habitats to grow and mature in marine environments.

4. STOCKING OF PARR AND SMOLTS AS A MANAGEMENT TOOL

The two previous sections have showed that several natural and anthropogenic factors can decrease the seaward migration success of Atlantic salmon and brown trout smolts. This section focuses on stocking of hatchery-reared parr and smolts as a management practice to compensate for declines in salmonid populations. Specifically, it discusses different strategies to improve stocking success of hatchery-raised salmonids. This thesis includes two distinct studies (referred to as **paper III** and **IV**) investigating the possibility to improve and develop rearing methods of supplementation hatcheries that increase performance of hatchery fish in nature. In **paper III**, we examine the effects of rearing density on the seaward migration success of Atlantic salmon smolts in a natural stream. The underlying question of **paper IV** is whether sorting salmonids according to larval development rates in the hatchery offers a possibility to select fish with traits especially suitable for restocking purposes. The reasoning and key results of these studies are presented in this section.

4.1. Background for stocking and performance of hatchery-reared fish

Many populations of Atlantic salmon and brown trout have become extinct or severely reduced across their entire native distribution due to anthropogenic factors (Jonsson and Fleming 1993, Parrish et al. 1998, Sandøy and Langåker 2001, Rasmussen and Geertz-Hansen 2001, Russell et al. 2012, ICES 2013). In Denmark, virtually all rivers and streams (>98%) have been affected by human activities at some stage (Rasmussen and Geertz-Hansen 2001, Rasmussen 2006). The vast majority of streams have been regulated and canalized due to agricultural activities, resulting in destruction of spawning and juvenile habitats (Rasmussen and Geertz-Hansen 2001, Rasmussen 2006). Man-made barriers such as weirs and dams cause severe problems for the spawning and smolt migration of Atlantic salmon and brown trout in many catchments (Aarestrup and Jepsen 1998, Aarestrup and Koed 2003, Rasmussen 2006). Likewise, establishment of wetlands for nutrient removal from rivers can significantly reduce smolt survival during seaward migration (Koed et al. 2006, Kristensen 2012, Boel and Koed 2013). Other anthropogenic factors that represent serious threads for many populations on a global scale include hydropower development, acid precipitation, human introduction of parasites (e.g., *Gyrodactylus salaris* and *L. salmonis*), overexploitation, climate change, and pollution with organic matter, nutrients, heavy metals as well as pesticides (Sandøy and Langåker 2001, Thorstad et al. 2008, 2012a, Finstad et al. 2011, Harris et al. 2011, Johnsen et al. 2011; Russel et al. 2012; Section 2).

There exists several different management strategies for conserving and enhancing threatened or declining salmonid populations (Harris and Milner 2006, Jonsson and Jonsson 2009, 2011). It is beyond the scope of this thesis to cover all these strategies thoroughly. This issue have been well described elsewhere (e.g., Crozier et al. 2004, Jonsson and Jonsson 2009). As mentioned, the primary focus of this thesis is on stocking of hatchery-reared parr and smolts as a management tool.

However, it should be stressed that habitat restoration and enhancement should always be the preferred solutions to support and enhance salmonid populations (Harris and Milner 2006, Jonsson and Jonsson 2009, 2011). Habitat restoration can include removal of physical constructions such as weirs and dams, allowing anadromous populations to migrate freely between the sea and the spawning grounds (Jonsson and Fleming 1993, Thorstad et al. 2008, Jonsson and Jonsson 2009). Graveling provides an example of habitat enhancement that can increase salmonid populations with reduced spawning opportunities (Jonsson and Jonsson 2009, 2011). Management regulations can also be implemented to protect populations from overexploitation (e.g., regulations of recreational and commercial fisheries) and habitat alternations (Jonsson and Fleming 1993, Jonsson and Jonsson 2009, 2011, ICES 2013).

Over the last several decades, stocking of artificially produced salmonids from hatcheries has been a common practice for restoring and enhancing wild populations (Jonsson and Fleming 1993, Cowx 1999, Jonsson and Jonsson 2009, 2011). Hatchery-reared salmonids are also stocked to enhance recreational fisheries (Jonsson and Jonsson 2009). Large numbers of Atlantic salmon and brown trout are released annually in several nations through various stocking programs (ICES 2013). In Denmark alone, approximately 1.4 million brown trout smolts were released in 2013 (P. Geertz-Hansen, personal communication). Although brown trout dominates the stocking programs in Denmark, up to 100,000 Atlantic salmon smolts are currently stocked annually (S.T. Thomassen, personal communication). In addition, roughly 480,000 brown trout and 560,000 Atlantic salmon parr (half-year and one-year old combined) were stocked into Danish rivers and streams in 2013. Supportive stocking of parr and smolts is a relatively costly management activity (Jonsson and Jonsson 2009). Moreover, the efficiency of many stocking programs has been questioned because salmonids raised in captivity often adapt poorly to natural conditions, showing impaired performance and diminishing return rates when compared to their wild counterparts (Brannon et al. 2004, Fraser 2008, Jonsson and Jonsson 2009, Johnsson et al. 2014). Often, the post-release survival of hatchery-reared Atlantic salmon and brown trout is less than half than that of wild individuals of similar size (Jonsson et al. 1991, 2003, Jonsson and Jonsson 2009, 2011, Aarestrup et al. 2014). For instance, Saloniemi et al. (2004) showed that the survival of wild Atlantic salmon smolts were 4.5 times higher than that of hatchery-reared fish in a northern Baltic river. Similar results have been obtained for brown trout by Aarestrup et al. (2014), who reported that survival of wild smolts was 1.8 to 2.9 times higher than that of reared individuals during downstream migration in River Gudenaa and early marine migration in Randers Fjord.

Previous research shows that the poor survival of hatchery-reared fish in nature can be partly attributed to the artificial rearing conditions (Brännäs and Johnsson 2008, Jonsson and Jonsson 2009, Johnsson et al. 2014). The captive environment differs from the wild in several aspects as fish are kept at high density in barren tanks with low structural complexity, predators are absent, food is abundant and less variable, diseases are treated, environmental factors are relatively stable over the season, and aggressive encounters with conspecifics are usually more common (Latremouille 2003, Huntingford et al. 2006, Ashley 2007, Brännäs and Johnsson 2008, Johnsson et al. 2014). Additionally, hatchery fish are frequently disturbed by caretakers and repeated handling associated with routine husbandry procedures can increase stress levels (Huntingford et al. 2006, Ashley 2007). Because of these environmental conditions, hatchery-reared salmonids typically develop morphological, physiological, and behavioral traits that deviate considerably from those of their wild counterparts (e.g., Huntingford 2004, Jonsson and Jonsson 2006, 2009, Stringwell et al. 2014, Johnsson et al.

2014). Even if local populations are used to produce fish for stocking, standard hatchery conditions are generally insufficient to prepare fish for a life in nature (Jonsson and Fleming 1993, Brännäs and Johnsson 2008, Aarestrup et al. 2014, Johnsson et al. 2014). In addition, selection pressure in hatchery environments is relaxed, with the egg-to-smolt survival usually exceeding 80% (Reisenbichler et al. 2004). By comparison, only 1-5% of the fry survive the first few months in the wild (Elliott 1994, Jonsson and Jonsson 2011). Hence, the reduced performance and survival of stocked salmonids in nature may also be a result of protection from natural selection prior to release (Brännäs and Johnsson 2008).

4.2. Strategies for improving stocking success of hatchery-reared salmonids

4.2.1. Release procedures

Mortality of stocked salmonids is often particularly high shortly after release into nature (Brown and Day 2002, Thorstad et al. 2011b). One of the main causes of mortality is predation (Kekäläinen et al. 2008, Thorstad et al. 2011b). Following transport to the stocking location, fish are usually released directly into the river (Finstad and Jonsson 2001, Brown and Day 2002). This release procedure is commonly referred to as hard release. However, there is good evidence that the post-release survival of reared salmonids can be improved by allowing acclimation to the prevailing environmental conditions in within-river enclosures (e.g., net pens) before release (Cresswell and Williams 1983, Jonssonn et al. 1999, Brown and Day 2002, Finstad et al. 2003). Such so-called soft releases may also allow fish to recover from transport-induced stress prior to release (Strange and Schreck 1978, Finstad et al. 2003).

The seasonal timing and location of release are other important factors influencing stocking success of hatchery-reared salmonids (Finstad and Jonsson 2001, Aprahamian et al. 2003, Thorstad et al. 2012b, Karppinen et al. 2014). For instance, release of hatchery smolts upstream of reservoirs and weirs can increase mortality due to predation and delay seaward migration (Jepsen et al. 1998, Aarestrup and Koed 2003; see Section 2).

Body size of hatchery fish at release is another factor that may influence the likelihood of survival in the wild (Hansen and Jonsson 1989, Finstad and Jonsson 2001, Kallio-Nyberg et al. 2004, Saloniemi et al. 2004). It has been suggested that a large smolt size can compensate for reduced ability to adapt to natural conditions, possibly by limiting the number of potential predators and increasing downstream migration speed through areas of high predation risk (Kallio-Nyberg et al. 2004, Thorstad et al. 2007, Lacroix 2008, Serrano et al. 2009).

To summarize, it is important to consider different release strategies in order to increase stocking success of hatchery-reared salmonids. There is currently a lack of studies investigating effects of different release strategies on the seaward migration success of smolts and return rate of adult fish.

4.2.2. Improvement of hatchery rearing methods

Several studies have evaluated the potential for developing rearing methods that improve the performance of stocked salmonids in nature (review by Näslund and Johnsson in press, Brown and Laland 2001, Brown and Day 2002, Johnsson et al. 2014). For example, life skill training protocols such as exposure to live prey and potential predators during rearing have been proposed to facilitate development of foraging and antipredator behavior (Brown and Laland 2001, Brown and Day 2002, Brown et al. 2003). However, there is currently little empirical evidence that prior exposure to live prey and predators improve survival of parr and smolts following release into nature (Mirza and Chivers 2000, Brown and Laland 2001, Hawkins et al. 2007).

Recent studies have also explored the effects of enriched raring environments on behavioral development and post-release performance (Näslund and Johnsson in press). Several different types of enrichments have been tested and added to the hatchery tanks, including gravel, stones, submerged tree tops, plastic bags, artificial vegetation, cover camo-nets, and plastic tubing (Näslund and Johnsson in press). Environmental enrichment have been shown to decrease stress and aggression levels of salmonids during rearing (Näslund et al. 2013), facilitate development of behavioral life skills such as foraging abilities and predator avoidance response (Näslund and Johnsson in press, Brown et al. 2003, Roberts et al. 2011, Rodewald et al. 2011), and ultimately improve survival of smolts during seaward migration (Hyvärinen and Rodewald 2013). A number of other studies, however, have failed to demonstrate positive effects of environmental enrichment on cognitive development and post-release performance of juvenile salmonids (Brockmark et al. 2007, 2010, Tatara et al. 2008, Tatara 2009, Brockmark and Johnsson 2010). Hence, additional full-scale studies are required to assess if there is any general benefits in usage of environmental enrichment in supplementation hatcheries (Näslund and Johnsson in press, Hyvärinen and Rodewald 2013). For example, any positive effects on post-release performance should be weighed against potentially increasing production costs related to additional cleaning of the hatchery tanks (Näslund and Johnsson in press; Johnsson et al. 2014).

Lowering hatchery rearing density has generally been shown to increase post-release survival of reared salmonids (Brockmark and Johnsson 2010, Brockmark et al. 2010, Barnes et al. 2013, Kavanagh and Olson 2014, Johnsson et al. 2014). For instance, recent studies on brown trout parr showed that reduced rearing density facilitated development of foraging abilities and anti-predator behavior, resulting in increased growth and survival following release into nature (Brockmark and Johnsson 2010, Brockmark et al. 2010). Furthermore, brown trout reared at reduced density appear to become dominant in competition for food over conspecifics kept at conventional densities (Brockmark and Johnsson 2010). The underlying mechanisms for improved development of adaptive behavior at reduced rearing density remain speculative (Brockmark et al. 2010, Johnsson et al. 2014). However, it has been proposed that individual decision-making is facilitated at reduced densities because fish are more able to swim around freely in the hatchery tanks, whereas high density may promote schooling (Johnsson et al. 2014). Sensory overload and crowding stress are also thought to reduce ability to learn individual behavioral skills (Brockmark et al. 2010, Johnsson et al. 2014).

Rearing density often influence growth and fin condition, both representing important indicators of aggression and stress levels in hatchery environments (Ellis 2002, Latremouille 2003, Huntingford et al. 2006, Ashley 2007, Johnsson et al. 2014). In general, salmonids reared at high densities experience lower growth and higher degree of fin erosion relative to individuals kept at lower densities (Turnbull et al. 1998, Ellis 2002, Brockmark et al. 2007, Kavanagh and Olson 2014). Kavanagh and Olson (2014), for example, found that juvenile steelhead reared at low density were larger at release, had significantly better dorsal fin condition, and greater smolt-to-adult survival rate in nature when compared to individuals reared at higher densities. It should be noted that not all studies report positive effects of reduced rearing density on survival of hatchery-reared salmonids in nature (Hopley et al. 1993, Clarke et al. 2013). This might have to do with differences in rearing facilities (e.g., pond vs. raceways), species-specific responses to crowding stress, and specific environmental conditions during rearing and following release (Edwing and Edwing 1995, Tipping et al. 2004, Johnsson et al. 2014). Therefore, more research is warranted to clarify the effect of rearing density on the post-release survival of stocked salmonids.

Hatchery-reared salmonids for supplementary purposes are often released as smolts in rivers, shortly before or at the time of seaward migration of their wild conspecifics (Jonsson and Jonsson 2009). As the riverine migration success of the released smolts to marine habitats may contribute to determine the overall efficiency of supplementation programs, it is imperative to evaluate the effect of rearing density on smolt migration. Hence, an important objective of this thesis was to investigate whether rearing density influence the post-release migration success of Atlantic salmon smolts to the sea (see **paper III**). First-generation offspring (F1) of Atlantic salmon originating from the River Storå stock, Denmark, were either reared at conventional hatchery density (HD; 1,500 fish m^{-2}) or at one-third of conventional density (LD; 500 fish m^{-2}). Conventional hatchery density was set according to local hatchery practice at the Danish Centre for Wild Salmon (DCV), Randers, Denmark. During the main smolt migration period, 300 one-year old smolts from each density treatment were individually PIT-tagged and subsequently released in Gudsø Stream, approximately 3.2 km upstream of the stationary antenna station S1 (see Fig. 1).

The results of **paper III** showed that the downstream passage at the antenna station was significantly higher (16%) for smolts reared at reduced density (Fig. 3). None of the released smolts were caught during electrofishing surveys after the smolt migration period had finished, suggesting that all released fish migrated or died in the stream. There are several potential explanations for the reduced migration success of smolts reared at conventional density. First, the primary cause of mortality in the stream was probably due to predation by grey heron, cormorants, and adult trout, which are all common in the area. As discussed above, reduced rearing density may promote development of adaptive behavior (Brockmark and Johnsson 2010, Brockmark et al. 2010, Johnsson et al. 2014). Hence, it is possible that deficits in development of antipredator behavior increased mortality of HD fish during downstream migration. Second, subsamples of fish taken from the rearing tanks demonstrated a higher degree of dorsal fin damage among individuals in HD group. Since aggressive encounters with conspecific are the primary cause of dorsal fin erosion in hatcheries (Abbott and Dill 1985, Turnbull et al. 1998, Latremouille 2003), this observation suggests increased levels of agonistic behavior in the HD treatment group. Agonistic interactions can be a major source of stress in hatchery environments (Schreck 1982, Wedemeyer 1997, Näslund et al. 2013), and it is possible that elevated levels of stress reduced the post-release migration success of smolts reared at conventional density (see **paper I**; Section 3).

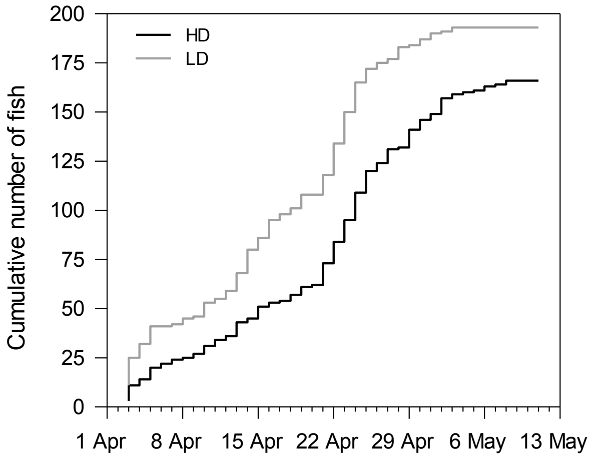

Figure 3. The cumulative migration success (%) in Gudsø Stream for Atlantic salmon (*Salmo salar*) smolts reared at conventional hatchery rearing density (HD) and one-third of conventional density (LD). Fish were released in the stream on 3 April 2013.

For instance, it is likely that elevated stress levels increase risk of predation (Olla et al. 1992, 1995; see Section 3). Third, the degree of opercula erosion was more severe in the HD treatment group. Damaged fins and opercula can probably reduce swimming performance and increase vulnerability to predation and pathogens during seaward migration (Arnold et al. 1991, Ellis 2002, Petersson et al. 2013). Finally, some studies suggest that rearing density may influence the degree of smolt development (Schreck et al. 1985, Brockmark et al. 2007). Given that fish reared at reduced density were at a more advanced stage of smoltification compared to conspecifics kept at conventional density, this could provide a potential explanation for the higher migration success of LD individuals. Additional research is required to unravel the ultimate mechanisms underlying the increased migration success of smolts reared at reduced density.

Overall, the results of **paper III** suggest that lowering rearing density can increase welfare in rearing (based on improved fin and opercula conditions) and optimize stocking success. Density reduction is a simple method to practice in supplementation hatcheries. However, before implementation further studies are needed to investigate the generality of the present findings. In addition, lowering hatchery rearing density may be economically challenging unless increased return rate of adults will compensate for the increased production cost per smolt. Answering this question require long-term studies elucidating the smolt-to-adult return ratio and development of cost-benefit analyses. Since consumers and stakeholders are becoming increasingly aware of and concerned about fish welfare during rearing, it is important that ethical issues are incorporated in these analyses (Huntingford et al. 2006, Ashley 2007).

4.2.3. Do emergence time and social rearing environment alter post-release performance?

In wild populations of salmonids, the period during which the fry emerge from the spawning nest and commence exogenous feeding is regarded as one of the most critical niche shifts in the life cycle (Elliott 1994, Jonsson and Jonsson 2011; see Section 1). The time to emergence from the gravel may vary by several weeks among individuals in the same spawning nest (Gustafson-Marjanen and Dowse 1983, Garcia de Leaniz et al. 2000). Previous studies suggest that the timing of this event is linked to individual physiological and behavioral traits (Metcalfe and Thorpe 1992, Metcalfe et al. 1995, Vaz-Serrano et al. 2011, Andersson et al. 2013, Thörnqvist et al. 2015). Individuals that emerge first from the nest appear to be more aggressive and bold compared to those emerging late (Metcalfe and Thorpe 1992, Vaz-Serrano et al. 2011, Thörnqvist et al. 2015). In addition, Metcalfe et al. (1995) showed that early emerging Atlantic salmon fry had higher metabolic rate and increased propensity of becoming socially dominant when compared to later emerging conspecifics. As a consequence, a higher proportion of early emerging Atlantic salmon fry reached body size threshold for smoltification during their first summer in the hatchery (Metcalfe and Thorpe 1992). Similar results were reported by McCarthy et al. (2003), showing a positive relationship between larval development rate and growth trajectories of Atlantic salmon in the hatchery. Taken together, these studies suggest a coupling between larval development rate, behavior, and performance under hatchery conditions. However, it remains unknown if such relationships persist in nature, where selection intensity is higher due to competition, disease, predation, and resource availability.

The question is whether sorting larvae according to development rate in the hatchery offers a possibility to select fish with traits especially suitable for stocking purposes? For instance, if it turns out that early emerging fry have higher post-release survival or propensity for smoltification than those emerging late, the former individuals may be selected for stocking. This could ultimately increase the effectiveness of supplementation hatcheries. This question was indirectly addressed in **paper IV**. More specifically, we investigated if emergence time of Atlantic salmon is related to post-release growth, survival, and migration status (resident or migrant). At the same time, there is an increasing awareness that the social rearing environment during early ontogeny may influence behavioral development and growth trajectories. Studies on rainbow trout, for example, suggest that previous conspecific experience may alter the degree of boldness and aggression of the individual (Frost et al. 2007, Sloman and Baron 2010). In addition, Pottinger (2006) found that growth of two lines of rainbow trout selected for divergent stress responsiveness (e.g., post stress plasma cortisol) was dependent on the social rearing conditions. When the two lines were reared together, the low responsive line (LR), characterized by being bold and aggressive, outgrew the timid and less socially competitive high responsive line (HR). However, no difference in growth was evident between the lines when LR and HR individuals were reared in separate hatchery tanks (Pottinger 2006). Based on these findings, it can be predicted that the social rearing environment influences behavioral development and post-release performance of Atlantic salmon with different emergence times.

The fish used in **paper IV** were F1 offspring of Atlantic salmon originating from River Dalälven in central Sweden. Eyed eggs were placed into artificial spawning nests that allowed fry to be sorted with respect to emergence time in the hatchery. Golf balls served as bottom substrate to mimic natural gravel. During the emergence period, fry were daily removed from the spawning nests and divided into three groups according to emergence time from the artificial gravel: early (first one-third), intermediate (middle one-third), and late (last one-third). To evaluate potential effects of the social rearing environment on behavioral development and post-release performance, these three emergence groups were reared separately or together in hatchery tanks for four month post-emergence. Next, the individual behavior of Atlantic salmon from each of the six treatment groups was characterized by use of three different behavioral assays: a basal locomotor activity test, a boldness test, and an escape response test during restraining in an emerged net. Following behavioral characterization, fish were PIT-tagged and released into a closed near-natural experimental stream in late autumn to compare growth, survival, and migration status among the treatment groups. A tube supplied the stream with river water from a nearby-electric dam in River Dalälven. Fish only had access to naturally occurring food items, and were exposed to natural predation from American mink and grey heron. The stream has a length of 110 m and a Wolf trap is situated at the lower end. This set-up allowed us to determine the number of out-migrating smolts the subsequent spring from each treatment group. The trap was inspected on a daily basis throughout the study. Length and mass of descending fish were noted. In June, seven months after fish were released into the stream, it was drained slowly to measure fish that remained in the system.

Results from the behavioral assays showed that the duration of escape attempts in the net differed between emergence groups, with intermediate individuals exhibiting longer escape duration compared to early emerging conspecifics. This suggests that individuals with an intermediate emergence time have higher physical stamina than those emerging early. By contrast, the social rearing environment had no significant effects on individual behavioral expression. Survival, growth, and number of out-migrating smolts in the stream were not correlated with emergence time or social rearing environment. Furthermore, despite individuals displayed extensive variation in basal locomotor activity, boldness, and their escape response during net restraining across treatment groups, none of these behaviors were associated with survival, growth, or migration status in the stream. Hence, our results support the view that fitness (i.e., growth and survival) and life-history predictions from laboratory measures of behavior should be made with great caution (see Adriaenssens and Johnsson 2009, Conrad et al. 2011, Mittelbach et al. 2014).

To come back to the main question: can sorting by larval development rate be utilized to select fish with trait especially suitable for stocking purposes? The short answer to this question is: probably not. Neither emergence time nor the social rearing environment influenced the post-release performance of the juvenile Atlantic salmon under near-natural conditions. Growth and survival of fish were also similar between treatment groups during rearing in hatchery prior to release. Even if a specific emergence group showed improved post-release performance, this relationship might depend on specific environmental conditions (e.g., intensity of predation and competition). Moreover, the long-term consequences of artificially selecting individuals according to development rate remains uncertain. Therefore, **paper IV** highlights the challenges associated with incorporating individual variation in bahaviour and larval development rate into fisheries management and conservation.

5. METHODS FOR STUDYING SMOLT MIGRATION

In recent decades, the development of electronic tracking technology (also known as biotelemetry) has greatly advanced our understanding of fish behavior and ecology (Cooke et al. 2013, Klimley 2013, Thorstad et al. 2013). Electronic tags that have been successfully used for studying migration behavior of juvenile salmonids include PIT tags, data storage tags (DST), radio tags, and acoustic tags (Jepsen et al. 1998, Aarestrup et al. 1999, 2003, 2014, Koed et al. 2002, Reddin et al. 2006, Thorstad et al. 2007, del Villar-Guerra et al. 2014). It is beyond the scope of this thesis to discuss the advantages and limitations of all these types of electronic tags. These aspects have been thoroughly addressed in a number of recent reviews which are highly recommended (Lucas and Baras 2000, Thorsteinsson et al. 2002, Cooke et al. 2013, Thorstad et al. 2013). Rather the information presented in this section focus on PIT telemetry since it was a critical component of this thesis. In **papers I** to **III**, hundreds of juvenile Atlantic salmon and brown trout were tagged with 23 mm PIT tags to identify individual growth, survival, and migration success of fish in different treatment groups. In **paper IV**, Atlantic salmon were tagged with 12.5 mm PIT tags.

The underlying assumption of biotelemetry studies is that the tagging procedure and additional burden of carrying the tag do not alter the behavior, growth, and survival of the tagged individual. Hence, an important aspect in many PIT telemetry studies is the choice of tag size. Larger PIT tags (e.g., 23 to 32 mm) have a higher detection range, but smaller tags (e.g., 8 to 12 mm) open the possibility to tag smaller individuals. While several studies have evaluated the effects of implanting 12 mm PIT tags in juvenile salmonids, comparable little information is available on the potential impacts of 23 mm PIT-tagging. Furthermore, the greater detection range of 32 mm PIT tags makes them attractive for many telemetry applications, but no previous studies have tested the effectiveness of these tags for marking smaller fish. We therefore conducted a laboratory study that aimed to evaluate the feasibility of 23 and 32 mm PIT tags as an individual marker in juvenile Atlantic salmon (referred to as **paper V**). The results of **paper V** are synthesized and put into perspective at the end of this section. First, I will discuss the advantages and limitations of PIT telemetry.

5.1. PIT telemetry

PIT telemetry was developed in the mid-1980s (Gibbons and Andrews 2004). Since then, this technology has been used extensively in fisheries research and greatly expanded our insights into fish behavior and ecology (e.g., Aarestrup et al. 2003, Zydlewski et al. 2006, Svendsen et al. 2007, Skov et al. 2008, Chapman et al. 2011, Cooke et al. 2013). PIT tags typically have a cylindrical shape and consist of a small glass-encapsulated electromagnetic coil and encoded microchip. The tag does not contain batteries and remain passive until it is energized by an electromagnetic field send from a detector. The operational time of PIT tags is therefore expected to last for the lifetime of the tagged fish, making this technique ideal for long-term studies (Gibbons and Andrews 2004, Cooke et al. 2013, Thorstad et al. 2013). When the tag is activated, it transmits a unique serial number to the reader (Lucas and Baras 2000, Lucas and Baras 2001, Gibbons and Andrews 2004). In theory, this feature permits unique identification of an infinite numbers of individual fish. Another key advantage with PIT tags are their small size, usually measuring between 8 to 32 mm in length, and 1 to 4 mm in diameter, enabling researchers to study migration of smaller fish than is possible with acoustic and radio telemetry (Cooke et al. 2013). Furthermore, PIT tags are inexpensive when compared to other electronic tags, facilitating large sample sizes (Gibbons and Andrews 2004, Zydlewski et al. 2006, Cooke et al. 2013, Thorstad et al. 2013). PIT tags are normally implanted into the peritoneal cavity of juvenile salmonids, either with syringe injectors or by making a small incision using a scalpel and inserting the tag manually (Lucas and Baras 2001, Gries and Letcher 2002, Thorstad et al. 2013). In **papers I** to **IV**, the PIT tags was inserted through an incision made slightly anterior to the pelvic fins on the ventral surface using a scalpel. The tagging incisions were left to heal without using closure materials (e.g., suture and tissue adhesive). In adult salmonids, it might be advantageous to inject the PIT tag in the dorsal musculature to prevent tag loss during spawning (Prentice et al. 1990, Bateman et al. 2009, Meyer et al. 2011). It only takes a few seconds for experienced surgeons to implant the tag into the peritoneal cavity or musculature of the fish. As such, PIT-tagging is easy to manage in field or laboratory applications with minimal handling time.

The primary limitation of PIT tags is their relatively short detection range, which is usually less than 200 cm perpendicular to the antenna (Zydlewski et al. 2006, Burnett et al. 2013, Cooke et al. 2013). As a consequence, PIT telemetry has largely been restricted to laboratory applications or smaller natural systems (Lucas and Baras 2001, Cooke et al. 2013, Thorstad et al. 2013). PIT tags can nevertheless provide valuable information about growth, survival, and migration of individual fish in larger systems when used in conjunction with the mark recapture method (Ombredane et al. 1998, Lucas and Baras 2000, Hedger et al. 2013). However, the temporal resolution in such studies is often poor and only provides information of change in location since previous captures, thereby underestimating the actual movement of fish (Lucas and Baras 2000). Obviously, this method also requires recapture of tagged fish, which is not always possible or suitable (Lucas and Baras 2000). PIT tags can also be used in combination with automated stationary scanning systems with tubular, square-shaped, or flatbed antennas (Armstrong et al. 1996, Lucas et al. 1999, Greenberg and Giller 2000, Barbin Zydlewski et al. 2001, Aarestrup et al. 2003, Johnston et al. 2009, Thorstad et al. 2013). These systems simply require that the tagged fish swim through the detection field, making recapture unnecessary. When a tagged fish is inside the electromagnetic field, the reading unit automatically stores the ID number of the tag with the time and date of detection on a memory card (Zydlewski et al. 2006). Owing to the limited detection range, the antenna arrays often have to be placed strategically at narrow passageways, such as fish passes (Prentice et al. 1990, Castro-Santos et al. 1996, Lucas et al. 1999). Cross-river antennae that scan the entire water column have also been successfully deployed in smaller river systems (Greenberg and Giller 2000, Barbin Zydlewski et al. 2001, Aarestrup et al. 2003, Ibbotson et al. 2004, Svendsen et al. 2007, Skov et al. 2008; **papers I** to **III**). Two or more consecutive antennas placed a few meters apart from each other allow researchers to determine the direction of fish movement (Greenberg and Giller 2000, Zydlewski et al. 2006, Skov et al. 2008). The two stationary PIT stations (S1 and S2; see Fig. 1) used in **papers I** to **III** each consisted of two square-shaped antennas spaced ~5 m apart, covering the entire cross-section of the stream. The key advantage with stationary antenna systems is that it enables one to continuously monitor the passage of individual fish (Greenberg and Giller 2000, Zydlewski et al. 2006). In other words, the temporal resolution with stationary systems is high and ideally suited for long-term tracking of migrating fish species that have to pass the antenna array one or several times during their life-cycle. As such, this technology has provided a powerful tool for studying the seaward migration of Atlantic salmon and brown trout smolts (Svendsen et al. 2007; **papers I** to **III**). More recently, portable PIT detectors (e.g., backpack and boat mounted models) have been developed to locate PIT tags in smaller ponds and streams (Roussel et al. 2000, Cucherousset et al. 2005, Hill et al. 2006, Linnansaari and Cunjak 2007). This technology is particularly suitable for investigating fine-scale movements, microhabitat use, and spatio-temporal distribution of fish in shallow streams (Hill et al. 2006, Cooke et al. 2013). In addition, portable PIT telemetry has enabled researchers to estimate predation rates on small fish from piscivorous birds (e.g., heron and cormorants) by locating tags on land in bird breeding colonies and roosting sites (Collis et al. 2001, Boel 2012, Skov et al. 2014). The disadvantage of portable systems is the limited temporal resolution compared with stationary systems as they must be operated manually (Johnston et al. 2009).

A critical assumption when using stationary antenna arrays is that the probability of the tag being detected should be high (that is, >95%; Zydlewski et al. 2006). Based on the methods described in Zydlewski et al. (2006), the probability of tag detection was estimated as ≥94% for S1 in Gudsø Stream in **papers I** to **III** (Fig. 1). The antenna stations were operating continuously throughout the studies. Overall detection efficiency of S2 could not be estimated due to the absence of downstream detections (see Zydlewski et al. 2006). Detection efficiency depends on antenna power, tag orientation, operation frequency, overall size of the antenna coil, and interference from other devices (Lucas and Baras 2001, Zydlewski et al. 2006, Burnett et al. 2013, Thorstad et al. 2013). It should be noted that missed detections may occur if two or more tagged fish pass the reading station simultaneously (Castro-Santos et al. 1996, Greenberg and Giller 2000, Morhardt et al. 2000, Lucas and Baras 2001). Likewise, fish sitting inside the antenna coil may block for detection of other tagged fish as they pass the reading station (Greenberg and Giller 2000, Lucas and Baras 2000). Fortunately, the incidence of such detection errors appears to be low. In a study with juvenile brown trout, Greenberg and Giller (2000) reported that only 0.2% of the total number of recorded tags were unidentifiable due to tag collisions. However, it is plausible that the frequency of tag collisions might be higher in studies involving shoaling fish species. High traveling speed of tagged fish through the detection field has also been associated with decreased detection efficiencies (Castro-Santos et al. 1996, Greenberg and Giller 2000). Moreover, as noted above, it is well established that larger PIT tags have greater detection range than smaller ones when energized by external antennae, everything else being equal (Zydlewski et al. 2006, Burnett et al. 2013). Therefore, the use of smaller PIT tags (e.g., 12 mm) has mainly been restricted to laboratory applications, mark recapture studies, or natural systems with water depths less than 40 cm (Brännäs et al. 1994, Ombredane et al. 1998, Cucherousset et al. 2005; **paper IV**). Larger tag sizes, such as 23 or 32 mm, offer a detection range up to100 cm, extending their applicability for certain telemetry applications (Morhardt et al. 2000, Roussel et al. 2000, Aarestrup et al. 2003, Linnansaari and Cunjak 2007; **papers I** to **III**).

As intra-coelomic implantation of PIT tags can adversely affect fish (Baras et al. 2000, Tatara 2009), researchers must often balance the trade-off between detection range and tag size, especially in investigations involving small-bodied individuals. Many telemetry studies follow the general rule of thumb proposed by Winter (1983), who recommended that the tag weight should not exceed 2% of the fish’s total body mass in air. However, several researchers have challenged the 2% rule and show that heavier tags not necessarily cause adverse effects on physiology and performance of juvenile salmonids (Brown et al. 1999, Connors et al. 2002, Jepsen et al. 2005, Smircich and Kelly 2014). For instance, a recent study on brook trout (*Salvelinus fontinalis*) found that intraperitoneal implantation of dummy acoustic tags weighing up to 7% of the fish’s body mass had no effect on mortality or swimming performance and there was no evidence of chronic stress related to carrying the tag (Smircich and Kelly 2014). Similarly, acoustic transmitters composing between 7 to 8% of the fish’s body mass did not adversely influence growth, survival, and swimming performance of coho salmon pre-smolts 300 days post-tagging (Chittenden et al. 2009). The effects of smaller PIT tags (i.e., 11 to 12 mm) on growth and survival of salmonids have generally been negligible and many studies conclude that individuals down to about 55 mm in total body length can be successfully tagged (Lucas 1989, Ombredane et al. 1998, Gries and Letcher 2002, Acolas et al. 2007, Richard et al. 2013). Sigourney et al. (2005), however, found negative effects of 11.5 mm PIT tags on survival and growth on Atlantic salmon parr 60 to 69 mm FL. Based on the results reported in the latter study, the lower size limit of 12.5 mm PIT-tagged Atlantic salmon was set to 85 mm FL in **paper IV**. Although this size limit may appear rather conservative, it was critical that the tag did not adversely influence the behavior, growth, and survival of the experimental fish (**paper IV**).

Previous studies have also examined the efficacy of 23 mm PIT tags among different species of juvenile salmonids (e.g., Zydlewski et al. 2003, Hill et al. 2006, Bateman and Gresswell 2006, Bateman et al. 2009). For instance, Bateman and Gresswell (2006) evaluated the effects of surgically implanted 23 mm PIT tags on growth and survival of steelhead 73 to 97 mm FL. The authors showed a transient slowdown in growth and higher mortality of tagged steelhead when compared to control and sham-operated (i.e., fish received surgery but no PIT tag was implanted) individuals (Bateman and Gresswell 2006). By contrast, Zydlewski et al. (2003) concluded that intra-coelomic implantation of 23 mm PIT tags provide a feasible method for marking steelhead 100 to 150 mm FL, with negligible effects on growth and survival. Using the same sized-tag and surgical technique, Roussel et al. (2000) examined the efficiency of a portable backpack reading unit to study diurnal movements and habitat preference of Atlantic salmon parr 64 to 94 mm FL in smaller streams. In the laboratory, the authors found a mortality rate of 21% among the tagged Atlantic salmon. However, no control or sham-operated group was included in the study, sample size was small (n = 33), and growth was not evaluated as an endpoint. Hence, there is a need for additional evaluation and clarification of whether and to what extend 23 mm PIT tags influence growth and survival of juvenile salmonids. Further, to our knowledge, no previous studies have investigated the efficacy of 32 mm PIT tags in small fish. We therefore conducted a laboratory experiment to evaluate the effects of 23 and 32 mm PIT tags on growth and survival of juvenile Atlantic salmon (see **paper V**). Experimental fish were hatchery-reared F1 offspring of wild Atlantic salmon originating from River Storå, Denmark. Atlantic salmon of three different size classes (I: 80-99 mm FL, II: 100-119 mm FL, III: 120-135 mm FL) were allocated to each of five treatment groups: control (no surgery or PIT tag implantation), sham-operated (surgery without PIT tag implantation), 23 mm PIT implantation with or without absorbable suture closure of the tagging incision, and 32 mm PIT tag implantation without suture closure of the tagging incision. The 23 mm PIT tags were surgically implanted through a 3 to 4 mm ventrolateral incision made slightly anterior to the pelvic fins using a scalpel. It was necessary to insert the 32 mm PIT tags posterior to the pelvic fins as the peritoneal cavity of the smaller individuals was very limited in its length to accommodate this tag size. The treatment groups were reared in hatchery tanks for 35 days, such that each tank contained fish from all treatment groups and size classes.

Mortality only occurred among Atlantic salmon tagged with 32 mm PIT tags. The mortality rate was 14% and was mainly observed among individuals ≤84 mm FL. External and internal examination of the dead fish revealed no evidence of accidental cuts or punctures to the internal organs from the scalpel and the tagging incisions were free of tissue inflammation as well as fungal infection. However, fish that died exhibited impaired swimming performance up to 4 days prior to death. More specifically, the tail beat frequency was elevated and irregular and fish showed symptoms of buoyancy problems, suggesting that the swim bladder of these individuals was not able to support the additional weight of the 32 mm PIT tag (0.8 g in air). Furthermore, since the body cavity of the smaller individuals was limited in its capacity to accommodate the 32 mm tag, it is likely that the tag inhibited normal swimming movements. Thus, fish presumably died from exhaustion and stress associated with carrying the tag. In a narrow body cavity, pressure from the tag on vital body organs also offers a plausible explanation for the observed mortalities.

For size class I, the average growth of mass was lower for fish tagged with 23 and 32 mm PIT tags without suture closure of the incision when compared to control, sham-operated, and 23 mm PIT-tagged individual with suture closure (Fig. 4). For size classes II and III, only growth of Atlantic salmon tagged with 32 mm PIT tags differed from that of the other treatment groups (Fig. 4). Depressed growth rates have frequently been reported post-tagging, but there appear to be much variation in the extent and duration of this impact (Baras et al. 2000, Bateman and Gresswell 2006, Acolas et al. 2007). In this respect, a number of studies on salmonids have shown that tagged individuals can compensate for reduction in growth within 1-2 months after PIT-tagging by increasing growth rates (Sigourney et al. 2005, Bateman and Gresswell 2006, Acolas et al. 2007, Richard et al. 2013). Reduction in growth following tagging may be a result of increased energy expenditure of carrying the tag or physiological adjustment needed to accommodate it. Energetic costs associated with healing of the tagging incision and tissue encapsulation of the implanted tag also represent potential mechanisms that can restrict the amount of energy available for growth. However, growth of sham-operated fish was similar to that of control individuals in **paper V** (see also Lucas 1989, Moore et al. 1990, Bateman and Gresswell 2006; Fig. 4), and post-experimental dissection of the Atlantic salmon only revealed a few instances of tissue encapsulation of the tag.

Beyond the assumption that the tagging procedure and tag should have minimal effects on the tagged fish, it is crucial that the tag loss rate is low in telemetry studies. Retention rates of PIT tags in juvenile salmonides are usually above 90% (Ombredane et al. 1998, Gries and Letcher 2002, Hill et al. 2006, Bateman and Gresswell 2006), but there are a few exceptions (Acolas et al. 2007, Bateman et al. 2009). In **paper V**, tag retention rate of 23 mm PIT tags was 97% when the tagging incision was left to heal without suture closure and no tag loss occurred when suture was used to close the incision. Retention rate of 32 mm PIT tags was 69%, with smaller individuals having the highest probability of tag loss. A narrow peritoneal cavity and swimming movements of the body could cause the tags to be pushed out through the tagging incision. This observation is in agreement with results obtained in other tagging studies on juvenile salmonids, where the likelihood of tag loss usually decreases with fish size (Hill et al. 2006, Acolas et al. 2007). While tagging incisions without suture closures was well-healed, 31% of sutured incisions exhibited mild to moderate fungal infection around the incision and suture insertion site. This result adds to earlier observations that suture can act as an attachment site for fungal growth (Jepsen et al. 2002, 2008, Skov et al. 2005).

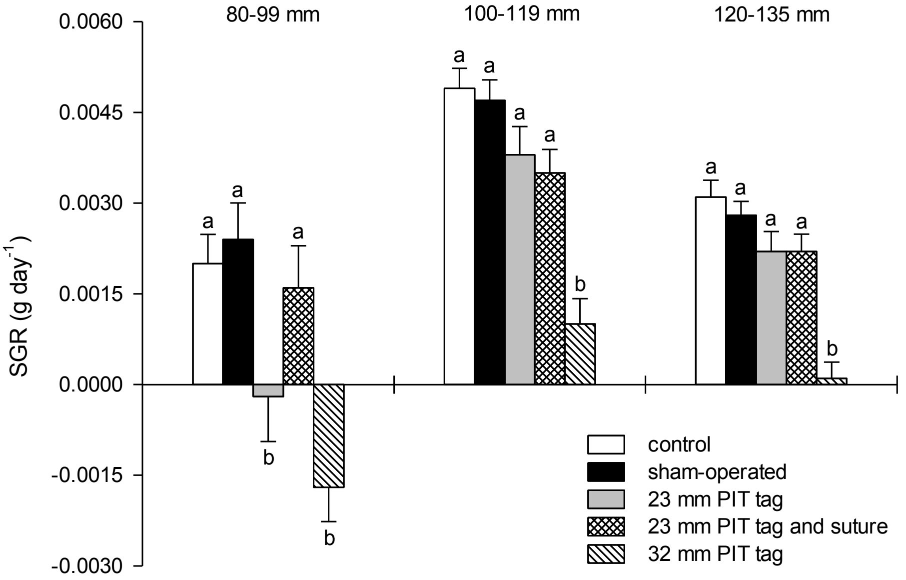

Figure 4. Specific growth rate (SGR; g day^{-1}) of Atlantic salmon (*Salmo salar*) in three different size classes (I: 80-99 mm FL, II: 100-119 mm FL, III: 120-135 mm FL) among five treatment groups: control (no surgery and PIT tag implantation), sham-operated (surgery without PIT tag implantation), 23 mm PIT (implantation of 23 mm PIT tag), 23 mm PIT tag and suture (implantation of 23 mm PIT tag and suture closure of the incision), and 32 mm PIT tag (implantation of 32 mm PIT tag). SGR of mass was calculated according to the following equation: $SGR = (\log_e M_f - \log_e M_i) \times t^{-1}$, where M_i is the mass at the time of tagging, M_f is the mass at experimental termination, and t is the time in days. Values are mean ± SE. Within each size class, bars not sharing the same letter are significantly different at $p < 0.05$.

Furthermore, 4% of the sutured incisions were partially open and highly inflamed and infected, resulting in an overall infection rate of 35%. It should be noted that other tagging studies have demonstrated that suturing can enhance the rate of incision healing and reduce tag loss rates (Baras et al. 1999, 2000, Roussel et al. 2000).

Based on the results of **paper V**, we recommend that 32 mm PIT tags should not be used in juvenile Atlantic salmon (80 to 135 mm FL) due to reduced growth and survival as well as high tag loss rate post-tagging. Surgical implantation of 23 mm PIT tags combined with suture closure of the incision did not influence growth and mortality of fish in any of the three size classes and tag retention was 100%. However, it is important to keep in mind that fungal infection and tissue inflammation only occurred when the incisions were sutured. Hence, additional studies are required to assess the potential consequences of fungal infection and inflammation on growth and survival of juvenile Atlantic salmon on a longer timescale before proper recommendations can be given. The growth was reduced for non-sutured fish implanted with 23 mm PIT in size class I (80 to 99 mm FL), but fungal infection was not observed when the incisions were left to heal without suture closure. In addition, tag retention rate was high for this treatment group (i.e., 97%). We therefore conclude that surgical implantation of 23 mm PIT tags without suture closure of the tagging incision provide as feasible method for marking Atlantic salmon 100 to 135 mm FL. The tag-to-body-mass ratio for these individuals ranged from 2.2 to 6% (mean: 3.5%). Hence, our study supports the view that the 2% rule should be regarded as rather flexible guideline (Brown et al. 1999, Jepsen et al. 2002, 2005, Smircich and Kelly 2014). In studies where growth is not a parameter of interest, 23 mm PIT tag implantation without suture closure may be useful in Atlantic salmon as small as 80 mm FL.

All brown trout and Atlantic salmon implanted with 23 mm PIT tags in **papers I** to **III** were ≥120 mm TL. Therefore, effects of 23 mm PIT-tagging on growth and survival of fish used in these studies are expected to have been negligible. We also believe that tag retention rates were high in our studies.

CONCLUSIONS AND PERSPECTIVES

The first part of the thesis focused on the impacts of cortisol manipulation on growth, survival, and seaward migration success of wild brown trout. The second part focused on developing and improving rearing method in supplementation hatcheries to increase stocking success of hatchery-reared Atlantic salmon. In the last part of the thesis, I tested the feasibility of 23 and 32 mm PIT tags in juvenile salmonids. A summary of the key finding presented in this thesis are detailed below as follows:

- In **papers I** and **II**, we investigated the effects of experimental elevation of blood plasma cortisol concentrations on wild brown trout in a natural stream. The findings of both studies suggest that chronic cortisol elevations can negatively affect growth, survival, and seaward migration success of juvenile brown trout. While this body of work has contributed to our understanding of the ecology of stress in wild salmonid populations, findings have also revealed several opportunities for further research. First, it was not possible to assess growth for trout that migrated from the stream to the sea. Since migratory trout tend to have higher metabolic rates than resident individuals, it is possible that growth would be even further depressed for migrating fish following exposure to an artificial stressor. Therefore, future studies should attempt to investigate growth of out-migrating trout by recapturing them along their migratory pathway to the sea. Second, it would be interesting to assess the impact of a chronic cortisol elevation on the anti-predator behavior of brown trout in laboratory settings. Such experiments together with post-treatment evaluation of disease burden and immune function could help unraveling the main factor responsible for the lower survival of the artificial stressed trout in the stream. Third, since the cocoa butter vector itself appeared to impact growth of trout, researchers should continue to develop and refine techniques for chronically increasing plasma cortisol titers in fish. For instance, it would be relevant to test the efficacy of mini-osmotic pumps, silastic tubing, and vegetable shortening as cortisol carriers in brown trout. Lastly, the long life span of the PIT tags will enable us to determine whether stressors experienced during the early life stages can cause long-term carryover effects on brown trout as they continue to grow and develop in the ocean and eventually return to the stream for spawning.

- Results of **paper III** showed that lowering conventional hatchery rearing density by one-third improved the post-release migration success of Atlantic salmon smolts to the sea. Lowering conventional rearing density will increase the production cost per smolt. To be economically feasible in full-scale commercial operations, it is therefore important that the higher costs in the hatchery are compensated by increased return rates of adult Atlantic salmon. Hence, an important task for future studies is to assess whether reduced rearing density increase the smolt-to-adult survival rate of Atlantic salmon. The findings of **paper III** also demonstrated that reduced density improved dorsal fin and opercula condition, implying that lowering rearing density can increase welfare of fish during rearing. Taken together, these results suggest that fisheries managers should focus on quality rather than quantity in further development of sustainable and ethically sound rearing methods.

- **Paper IV** aimed to investigate whether individual variation in emergence time from spawning nests of hatchery-raised Atlantic salmon fry and the early social rearing environment is linked to behavior and post-release performance. We only found little evidence that emergence time was correlated with behavioral expression. Moreover, results showed no effect of emergence time and rearing environment on the post-release growth, survival, and smolt status of the Atlantic salmon. Along these lines, although fish displayed extensive variation in individual behavior across treatment groups, this was not translated into difference in performance in the stream. Hence, our results support the view that the link between individual behavioral expression from laboratory measures and subsequent performance in nature is often flexible and probably depend on specific environmental conditions. The results also highlights the difficulties associated with incorporating variation in larval development rate into management practices.

- The objective of **Paper V** was to determine the efficacy of 23 and 32 mm PIT tags in juvenile Atlantic salmon. Surgical implantation of 23 mm PIT tags without suture closure of the tagging incision was found to be a feasible method for individual marking of Atlantic salmon 100 to 135 mm FL. At this point, we caution researchers about the use of sutures to close the surgical incisions due to high rates of fungal infection and tissue inflammation around the incision site. In addition, we recommend that 32 mm PIT tags should not be used in juvenile Atlantic salmon 80 to 135 mm FL. Further studies on larger

Atlantic salmon are warranted to establish a suitable size limit for using 32 mm PIT tags. Additional studies are also required to assess whether the results of the present laboratory study is applicable in natural systems. For instance, it is possible that effects of tagging hatchery-raised fish may be different from those of wild fish. Moreover, fish in hatchery environments are not exposed to the same stressors (e.g., predation, competition, density, pathogens and food availability) that fish experience in nature. Finally, since tagging effects may be species-specific, it would be interesting to evaluate the efficacy of 23 and 32 mm PIT tags in juvenile brown trout. Researchers should continue to validate and refine tagging techniques to ensure that the collected data are representative of untagged individuals.

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PAPER I

Does cortisol manipulation influence outmigration behaviour, survival and growth of sea trout? A field test of carryover effects in wild fish

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Wild brown trout (*Salmo trutta*) smolt. Photo: Martin Hage Larsen.

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Does cortisol manipulation influence outmigration behaviour, survival and growth of sea trout? A field test of carryover effects in wild fish

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ABSTRACT: For anadromous brown trout *Salmo trutta*, the transition from life in freshwater to the marine environment is an inherently challenging and dangerous period characterized by high levels of mortality. As such, smoltification is a relevant life-history phase to examine how physiological state, in particular glucocorticoids, influences fitness-oriented endpoints such as migration timing and survival. We experimentally assessed the effect of cortisol by combining passive integrated transponder (PIT) telemetry with a physiologically relevant exogenous cortisol manipulation (i.e. intracoelomic injection) in juvenile sea trout in the Gudsø Stream, Denmark. Individual survival, migration behaviour (timing and speed), and growth were assessed for 4 treatment categories: control (CO), sham (SH), and low- (LW; 25 mg kg^{-1}) and high-dose (HI; 100 mg kg^{-1}) cortisol. There was no difference in the timing of migration among treatments, but trout in the HI treatment had lower survival rates to the lower station (41.6%) when compared to the CO (53.9%) and SH (52.3%) groups. After migration, the system was electroshocked again to contrast growth of trout that remained in the system. HI, LW and SH individuals recaptured in the stream had lower growth rates for length than the CO treatments; HI and LW also had significantly lower growth rates for mass than CO trout. Future monitoring of this population may demonstrate the long-term repercussions of chronic stress as trout return from the ocean. This study provides contributions to our understanding of the relationship between organismal condition and fitness while elucidating the potential for carryover effects, i.e. lasting effects that influence future success.

KEY WORDS: Allostasis · Carryover effects · Migration · Stress

INTRODUCTION

Factors that influence the growth and survival of early life stages of fishes are critical for determining the long-term structure and viability of their population (Schlosser 1991). For anadromous brown trout *Salmo trutta* (hereafter called sea trout), the early phase of their life cycle occurs in freshwater tributaries that feed into the ocean. For this species and other salmonids, the pre-smolt and smolt life stages are an exceptionally important and sensitive period. Energy reserves are typically low, yet these fish are exposed to high levels of predation along their migratory route and must undergo a physiologically challenging transition from freshwater into a saline environment (Aarestrup et al. 2000, Jonsson & Jonsson 2011). Lasting effects such as body condition or parasite load from their experience on their natal grounds (e.g. carryover effects) may dictate the timing and success of their migration to the ocean and ultimately

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*Corresponding author: midwoodj@gmail.com their survival (Norris 2005, Harrison et al. 2011). Despite the potential for carryover effects, little work has been done to determine how environmental conditions in natal rivers may affect sea trout as they transition to the ocean (Jonsson & Jonsson 2011).

Wild populations of fish are subject to a wide variety of both anthropogenic and natural challenges that can range from acute stressors such as predation attempts or fisheries interactions to chronic stressors like habitat alteration and pollution (Pickering 1989, Schlosser 1991, Fraser & Gilliam 1992, Wang et al. 2000). In teleost fishes, elevated plasma cortisol is a natural response to stress, with circulating levels providing a useful physiological indicator of a fish's stress level (reviewed in Iwama 1998). The corticosteroid pathway helps mobilize energy reserves in response to a stressor, thereby increasing metabolic activity (reviewed in Mommsen et al. 1999). While these changes increase the amount of energy available in order for an individual to respond to an immediate stressor, they also reduce the amount of energy available for essential functions such as growth and reproduction (Redding et al. 1986, Carragher et al. 1989, Gregory & Wood 1999, Fullerton et al. 2000). The stress response axis also plays an important role in allostasis or adaptation to novel environmental conditions such as those caused by an acute human disturbance. While short-term allostasis may allow fish to survive this type of environmental perturbation, the carryover effects of activation of the stress axis may also reduce long-term fitness (Schreck 2010, Harrison et al. 2011) as observed in studies of compensatory growth (Metcalfe & Monaghan 2003).

Intra-coelomic injection of cortisol is a common method for artificially increasing plasma cortisol in teleost fishes, albeit mostly in a laboratory context (Pickering & Duston 1983, Pickering 1993, Gamperl et al. 1994, Hoogenboom et al. 2011). Using this technique, early laboratory experiments were instrumental in elucidating the mechanisms behind an individual's response to stress (reviewed in Barton & Iwama 1991, Pickering 1993); however, there is still a need to apply this understanding to wild populations to put such work into an ecological context. Similar to laboratory studies, exogenous cortisol manipulation in wild populations of fish enables an experimental approach with both treatment and control groups. This approach was used recently to document a carryover effect (decreased survival compared to a control) in largemouth bass *Micropterus salmoides* that were treated with cortisol and released into their natural environment when exposed to a temporally separated second stressor (i.e. winter hypoxia; O'Connor et al. 2010). An integral component of this study was the combined use of a physiological manipulation (cortisol injection) and biotelemetry. Together, they enabled the researchers to monitor the individual movements and behaviour of both treated and control fish in a natural setting.

Biotelemetry offers one of the best options for monitoring a fish's response to an external perturbation in a natural setting (Cooke et al. 2004). This technology allows for the monitoring of individual movements and behaviour and, while radio or acoustic tracking can provide accurate movement information for individuals, they are cost prohibitive for applications that involve large numbers of fish. Passive integrated transponder (PIT) tags are a comparatively inexpensive and an easily applied option that also provide individual coding for each fish, have minimal effects on growth and survival, and have a very long life span (Zydlewski et al. 2001, Gries & Letcher 2002). By combining these tags with cross-stream PIT scanning arrays, long-term continuous data on individual movements can be acquired (Zydlewski et al. 2001, Gibbons & Andrews 2004).

Despite their potential ecological importance, there is a shortage of studies that document the response of wild fish to stress in their natural environment and its potential for carryover effects (Cooke & O'Connor 2010, O'Connor et al. 2011, Nagrodski et al. 2013). For sea trout, little work has been done to understand relationships between ecology (e.g. population-level processes) and physiology (e.g. organismal condition and health) in a natural setting (Jonsson & Jonsson 2011), especially during early life-stages. Our goals were therefore to combine PIT telemetry with a cortisol manipulation to experimentally assess the impact of a chronic stressor on the individual growth, survival, and migration behaviour of wild, free-swimming sea trout. In sea trout, chronic stressors are known to impair growth and since migration timing is linked to an individual's size, this stressor may also delay their migration. We therefore predict that stressed individuals will experience impaired growth, which will result in delayed migration relative to controls. We also predict that stressed individuals will not be as successful at undertaking the physiologically demanding migration, resulting in lower survival rates for the stressed treatments.

MATERIALS AND METHODS

Study location and fish sampling

Brown trout smolts exhibit partial migration, whereby a portion of the population migrates to lakes or the

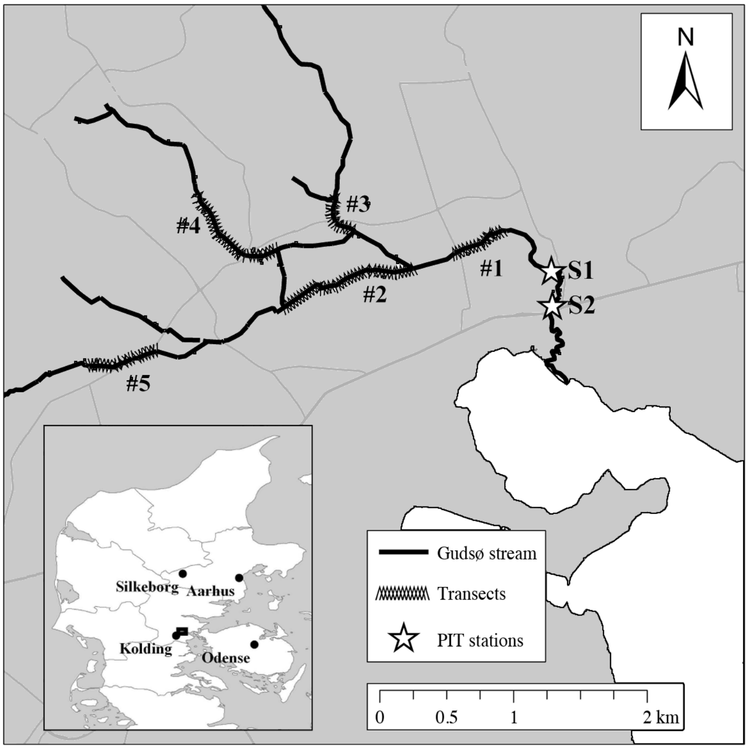

Fig. 1. Inset: location of Gudsø Stream (northeast of Kolding, Denmark; box). Main map: location within the stream of the 5 transects where trout were captured and the passive integrated transponder (PIT) stations (S1 and S2). A millpond is situated between S1 and S2 and serves as a potential population sink for migrating trout

ocean and the rest remain in their natal system and become residents (Jonsson & Jonsson 1993, Økland et al. 1993, Boel 2012). Polymorphs can co-exist because migrating trout have higher mortality rates but resident trout have lower fecundity (Jonsson 1985, Elliott 1993). Their individual condition and local resources in the late fall are thought to be the prime drivers behind smoltification, such that trout with lower food requirements tend to become residents, and those with higher requirements migrate from the river (Forseth et al. 1999, Cucherousset et al. 2005). We therefore opted to physiologically manipulate the trout after they had made the 'decision' to migrate or stay, to ensure that the stressor would not determine their smolt status (i.e. individuals in the different treatments were equally likely to migrate or become a resident).

The Gudsø Stream is located in east-central Jutland, Denmark, and flows through primarily agricultural areas before reaching the sea at Kolding Fjord (Fig. 1). The stream supports a natural population of brown trout as well as eel *Anguilla anguilla* and lamprey *Lampetra planeri*. There is an old mill with a dam and millpond located ~1 km upstream of the fjord. This landscape feature serves as a potential sink for out-migrating trout and is situated between our telemetry stations (see 'Biotracking' below). A fish ladder in this location provides both upstream and downstream access for migrating fish. Five regions of the Gudsø Stream upstream of the mill were sampled from March 6 to March 12, 2012 (Fig. 1). Transects 1 and 2 (0.4 and 1.1 km in length, respectively) were located on the main stem of the stream. Transects 3, 4, and 5 (0.8, 0.3, and 0.5 km length, respectively) are sub-tributaries that flow into the main stem of the stream. All trout greater than 120 mm in total length were collected using a backpack electroshocking unit (Scubla ELT 60 II GI; run at 300 volts) and kept in a 60 l container with fresh water.

PIT tag insertion and cortisol treatment

Trout were placed in a solution of 0.03 g l^{-1} benzocaine until their opercular rate became slow and they did not respond to external stimuli (3 to 4 min). This ensured that they were immobilized during the procedure. They were first measured (total length (TL) to nearest 1 mm) and weighed (wet body mass to nearest 0.1 g). Next, a small incision (~5 mm) was made on the ventral-left side of the body anterior to the pelvic fin. A 23 mm PIT tag (Texas Instruments, RI-TRP-RRHP, 134 kHz, 0.6 g mass in air) was inserted into the body cavity through the incision and the individual tag number was recorded. The tagging incisions were left to heal without suture closure. This approach was based on the standardized methods used for PIT tagging at the Technical University of Denmark and was performed in accordance with guidelines from the Danish Animal Experiments Inspectorate (License Number: 2013-15-2934-00808).

Immediately following PIT tagging, each trout was assigned into 1 of 4 treatment categories: control (CO), sham (SH), low-dose cortisol (LW), and high-dose cortisol (HI). Treatments were assigned in a stratified random design, such that an attempt was made to ensure that the average size of fish in each category was equal. Following PIT tagging, CO fish were allowed to recover in a 60 l container with fresh water until they were released (typically between 30 and 60 min). Fish in the remaining 3 treatments received either an injection of plain cocoa butter (SH) or a mixture of cocoa butter and cortisol prior to recovery (between 30 and 60 min). To make the LW and HI cortisol treatments, 0.25 g and 1.00 g of cortisol were added to 100 ml of melted cocoa butter, respectively. The cocoa butter treatments were reheated (~40°C) in the field to melt them and were shaken repeatedly throughout the administration process to prevent the cortisol from precipitating out of solution. Injections were made on the right ventral side of the body anterior to the pelvic girdle using an 18-gauge needle. The amount of cocoa butter or cortisol–cocoa butter given to each fish was dependent on the mass of the fish in a ratio of 0.01 ml of cocoa butter to 1.0 g of fish. This resulted in cortisol dosages of 25 mg kg^{-1} for the LW treatment and 100 mg kg^{-1} for the high treatment.

While the magnitude of the plasma cortisol response is known to vary among individuals in laboratory studies (Fevolden et al. 1991, Pottinger et al. 1992), average baseline values for brown trout have been determined to range from 2 to 5 ng ml^{-1} rising up to 100 ng ml^{-1} following an acute stressor. In contrast to this drastic short-term increase, chronic stressors may result in long-term increases of 10 to 20 ng ml^{-1} (Pickering et al. 1982). Pickering (1989) found that when using cocoa butter as a carrier, a dosage of 30 mg of cortisol per kg of body mass raised circulating plasma levels to 15 ng ml^{-1} or within the range of a chronic stressor. Our LW treatment is therefore designed to simulate a chronic stressor that should last for 2 to 4 wk (Pickering & Pottinger 1985). In keeping with previous studies that have found dosages of 50 mg kg^{-1} elevating cortisol levels for up to 5 wk (Pickering & Duston 1983), the HI treatment (4× the LW concentration) is designed to elicit a more extreme response.

Biotracking

Two antenna arrays, each consisting of a pair of in-stream antennas spaced ~5 m apart, were established near the bottom of the stream system (Fig. 1). The first station (S1) was located in the stream upstream of the millpond. The second station (S2) was located below the small fish ladder that connects the millpond to the downstream portion of the stream that flows to the ocean. This setup allowed us to determine when a fish entered the millpond (passed through S1) and when it exited in the seaward direction (passed through S2). The distance between the 2 stations is ~150 m. It was not feasible to track trout beyond this point because the lower reaches of the stream pass through an extensive marshland for ~1 km.

To determine the efficiency of our antennas, we calculated *in situ* efficiency, which has 2 assumptions: (1) the probability of being detected at the first array is independent of the probability of detection at the second array, and (2) tagged fish passing through the first array are moving in the direction of the second array. These assumptions were met by our array design such that the distance between the 2 arrays is adequate to make detection probabilities independent, and trout entering the millpond are likely migrating to the ocean and therefore moving in the direction of S2. Based on these assumptions, *in situ* detection efficiency was calculated as the proportion of fish passing S2 that were also detected at S1.

Growth measurements

Approximately 110 d after tagging, on June 25 and 26, 2012, the 5 transects in the Gudsø Stream were resampled using a backpack electrofisher (Fig. 1). Trout that were captured were anaesthetized and tagged fish were identified using a PIT tag scanner (Agrident, APR350) then measured as described above. Trout were allowed to recover in a container (40 l) with aerated stream water for 15 min and then released into the stream. The instantaneous growth rate (G) for both length and mass were calculated for all recaptured fish (Eq. 1; described in Schreck & Moyle 1990).

$$G = (\log_e Y_2 - \log_e Y_1)/(t_2 - t_1) \qquad (1)$$

Where Y_1 is the mass or length at the time of tagging (t_1) and Y_2 is their mass or length at the time of recapture (t_2). Both t_1 and t_2 are measured in days.

Statistical analysis

The total numbers of out-migrating fish detected at S1 and S2 were used as a proxy of migration survival. A chi-squared analysis was used to compare migration rates among treatments, with binary values for station passage (1) or no passage (0). It was assumed that trout that were detected at S2 also passed through S1.

ANOVA was used to compare the timing of migration (day of S1 and S2 passage), the time it took fish to reach S1 and S2 (days since tagging), and the initial mass, length and Fulton's condition factor (K) of fish in each treatment, where K = [mass (g) × 100]/[length (cm)^3]; Ricker 1975). Similarly, for fish recaptured in the stream, ANOVA was used to compare treatment effect on the instantaneous growth rate for both length (G_L) and mass (G_W). A post-hoc Tukey-Kramer analysis was conducted if ANOVAs were significant. Length and mass data were log transformed to equalize variance among treatments, except when used to calculate the instantaneous growth rate. A Fisher's exact test was used to compare both the number of instream recaptures relative to the total number of fish per treatment that did not pass a station and survival to S1 and S2 relative to the total number of treated fish between treatments. The variation reported with all values is given as standard deviation (±SD). All analyses were performed in JMP v. 9.0.2 (SAS Institute).

RESULTS

Tagging

In total, 771 trout were PIT tagged and treated in roughly equal numbers (Table 1). There were no significant differences among treatment groups in terms of initial mass (ANOVA, F = 0.056, df = 3, p = 0.982), length (ANOVA, F = 0.036, df = 3, p = 0.991), or Fulton's condition factor (ANOVA, F = 1.645, df = 3, p = 0.178; Table 1).

In-stream recaptures

A total of 96 trout were recaptured during the second sampling of the system that occurred on June 25 and 26. Roughly equal numbers of trout from the different treatment groups were recaptured (Table 2), with no significant difference in total number (Fisher's exact test, p > 0.300 for all combinations). In terms of instantaneous growth rate for mass, there was no significant difference between the CO and SH treatments (0.65 ± 0.19 g d^{-1} and 0.50 ± 0.20 g d^{-1}, respectively; ANOVA, p = 0.056; Fig. 2A). However, the LW and HI treatments had a significantly lower G_W than the CO treatment (0.48 ± 0.18 g d^{-1} and 0.41 ± 0.22 g d^{-1}, respectively; ANOVA, p < 0.001; Fig. 2A). For length-specific growth, all treated fish had significantly lower G_L than CO fish (ANOVA, p < 0.001; Fig. 2B). While not significant, there was a decreasing trend in G for both length and mass with the presence of cocoa butter and an increasing concentration of cortisol (Fig. 2). The length, mass, and condition factor did not differ among treatments (ANOVA, all p > 0.100; Table 2).

Table 1. *Salmo trutta*. Mean initial length, mass, and condition (Fulton's condition factor) for trout in the 4 different treatments. Based on an ANOVA, there were no significant differences among treatments for these parameters. Variation is given as SD

Treatment	No.	Length (cm)	Mass (g)	Condition factor (K)
Control	194	14.8 ± 2.4	30.6 ± 15.2	0.918 ± 0.059
Sham	195	14.6 ± 1.8	30.2 ± 12.2	0.925 ± 0.062
Low	192	14.6 ± 1.8	30.6 ± 13.1	0.933 ± 0.070
High	190	14.7 ± 2.0	30.7 ± 14.6	0.922 ± 0.074

Table 2. *Salmo trutta*. Recapture data from June 2012 for recapture length, mass, and condition factor (Fulton's condition factor K). Variation is given as SD. There were no significant differences among treatments

Treatment	No. recaptured	Length (cm)	Mass (g)	Condition factor (K)
Control	20	17.4 ± 2.2	56.5 ± 24.8	1.02 ± 0.06
Sham	22	17.2 ± 1.8	55.8 ± 18.0	1.07 ± 0.08
Low	28	16.8 ± 2.2	53.9 ± 24.2	1.08 ± 0.07
High	26	16.0 ± 1.9	45.7 ± 19.7	1.06 ± 0.09

Migration timing

In total, 445 and 381 trout were detected passing S1 and S2, respectively. Of the 381 trout that passed S2, 14 had not been previously detected at S1. This allowed us to estimate the detection efficiency of S1 as 96.3%. We were not able to calculate detection efficiency to S2. Trout were detected moving downstream from March 8 until June 8 (Days 68 and 160, respectively). There was a clear 'peak' in downstream migration that occurred from April 10 to April 13. The majority of tagged trout that passed through each station during the entire migration period were detected during that 4 d period (56.7% at S1 and 68.8% at S2). Neither the average day of passage at S1 and S2 nor the time (in days) to reach S1 or S2 was significantly different among treatment groups (ANOVA, all p > 0.100; Table 3).

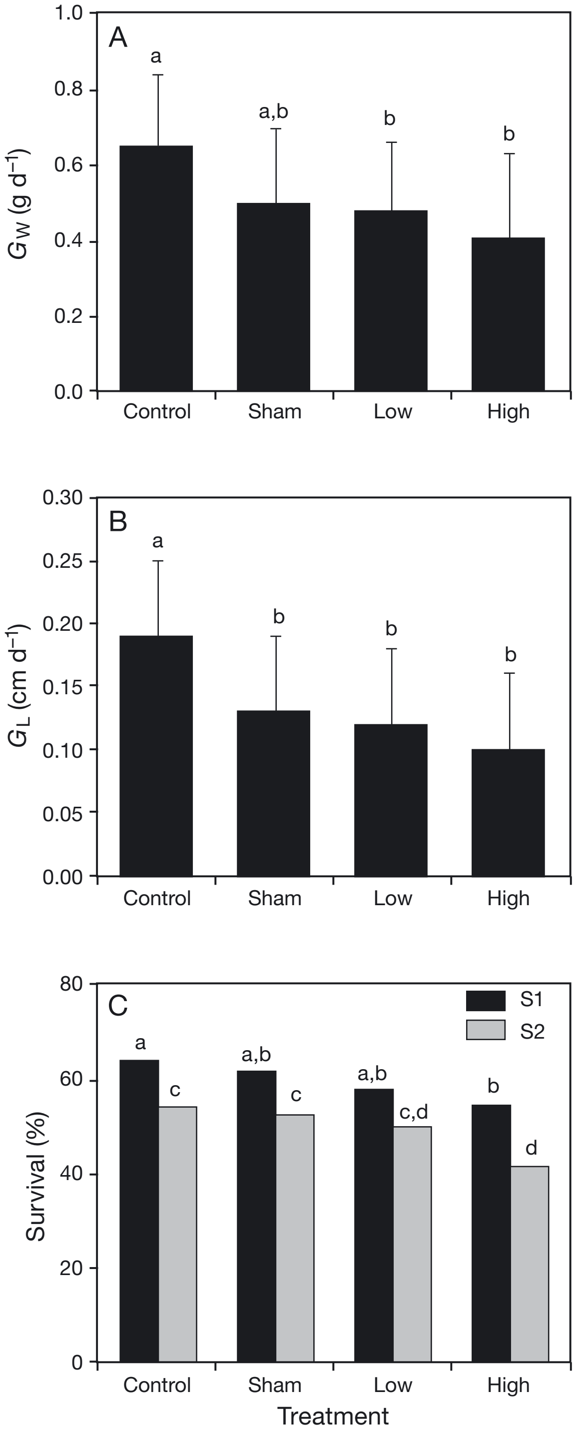

Fig. 2. *Salmo trutta.* Response of brown trout to 4 treatments: control (only PIT tagged), sham (PIT tagged and injected with cocoa butter), low-dose cortisol (PIT tagged and treated with a 25 mg kg^{-1} solution of cortisol) and high-dose cortisol (PIT tagged and treated with a 100 mg kg^{-1} solution of cortisol). Instantaneous growth rate for (A) mass (G_W; g d^{-1}) and (B) length (G_L; cm d^{-1}). (C) Migration survival (%) to PIT stations (S1 and S2) for the 4 treatments. Values are means ± SD; different letters show significant differences at $p < 0.05$

Survival to stations

Trout in the HI treatment had significantly lower migration survival to S1 and S2 than trout in the CO treatments (Fisher's exact test; p = 0.0303 and p = 0.0189, respectively; Table 3, Fig. 2C). Survival to S2 for HI treated fish was also significantly lower than SH trout (Fisher's exact test; p = 0.042). There were no significant differences in survival between the other treatments (Fisher's exact test; p > 0.100). Although not significant, there was a general trend towards lower survival with treatment and increasing cortisol concentration (Table 3, Fig. 2C).

DISCUSSION

Small-scale environmental changes can cause long-term or chronic levels of stress in fishes (Pickering & Stewart 1984). Therefore, modifications to the natural environment are a potential source of stress for juvenile trout in their natal habitat. Furthermore, the impact of these stressors on a trout's physiological state during its transition to the ocean has important implications for the long-term viability of a population and is critical for maintaining a productive fishery. Using a combination of PIT telemetry and cortisol manipulation, we undertook one of the first experiments to test whether chronic stressors have carryover effects on individual survival, out-migration behaviour, and growth of wild, free-swimming sea trout smolts. Broadly, there was a consistent decreasing trend for growth and survival based on the degree of the stress that was applied.

Growth

Carryover effects are known to occur in wild populations of brown trout where, despite a starved individual's ability to make up for a weight deficit, their survival is still depressed over the long-term (Johnsson & Bohlin 2006). Similarly, the long-term fitness of a fish can be depressed by cortisol manipulation (Gamperl et al. 1994, Schreck 2010). In this study, we observed a decrease in length- and mass-specific growth rate for recaptured trout that were part of the cortisol treatment groups (HI or LW) in comparison to the CO, but not compared to SH trout. Pre-smolt condition and growth in their natal freshwater streams is a critical period for ensuring a trout's long-term survival in the ocean, since larger fish entering the ocean often have higher survival rates (Rasmussen

Table 3. *Salmo trutta*. Timing of detection at PIT stations (S1 and S2) and the percentage of trout from each treatment that passed each station. Different superscript letters for the proportion of fish reaching S1 and S2 as well as the number moving between S1 and S2 denote significant differences between treatment groups based on a Fisher's exact test ($p < 0.05$). No letters signifies no differences among treatments. Variation is given as SD

Treatment	Initial no.	% reaching S1	No. of days to S1	Day of S1 passage	% reaching S2	No. of days from S1 to S2	Day of S2 passage	% moving from S1 to S2
Control	194	64.1^{a}	33.0 ± 10.8	101.4 ± 10.9	53.9^{a}	3.8 ± 5.7	104.8 ± 11.7	84.0
Sham	195	61.9^{ab}	34.9 ± 9.6	103.2 ± 9.5	52.3^{a}	3.4 ± 5.6	105.1 ± 9.0	84.2
Low	192	57.8^{ab}	36.0 ± 12.6	104.1 ± 12.3	50.0^{ab}	2.7 ± 5.7	106.1 ± 11.2	86.5
High	190	54.2^{b}	32.6 ± 11.2	100.9 ± 11.1	41.6^{b}	4.7 ± 7.2	103.0 ± 11.5	76.7
All Trout	771	60.0	34.1 ± 11.1	102.4 ± 11.0	49.4	3.6 ± 6.0	104.8 ± 10.9	83.0

1986, Bohlin et a. 1993, Jonsson et al. 1991). Therefore, the observed lower growth rates in treated fish (SH, LW and HI) are evidence of a carryover effect from the early spring treatment, suggesting that stressors in their natal habitat can potentially decrease their long-term fitness.

Johnsson & Bohlin (2006) documented compensatory growth in brown trout following a period of starvation. While we were unable to determine if such growth occurred in this study, given sufficient time it is possible that treated fish that experienced an allostatic load would eventually be able to recover such that their length and mass were similar to that of the control group. In Johnsson & Bohlin (2006), it took 1 mo for trout to compensate for impaired body condition, whereas for length, it took some individuals up to 5 mo. In the current study, resident trout were recaptured within ~3 mo, sufficient time for compensatory growth to improve their body condition. Unfortunately, we were not able to assess the instantaneous growth rates for trout that migrated from the system, although it is possible that growth would be even further depressed since migratory individuals tend to have higher metabolic rates than resident fishes (Forseth et al. 1999, Cucherousset et al. 2005). Future studies should attempt to assess the condition and growth rates of out-migrating individuals by re-capturing them along their migration pathway.

Migration timing

Despite the risks associated with migrating to the ocean, it is still beneficial for trout to undertake this migration because of the long-term benefit of increased reproductive capacity (Jonsson 1985). In freshwater tributaries, trout that grow faster and smoltify more quickly tend to migrate prior to smaller trout (Rasmussen 1986, Bohlin et al. 1993). Therefore, based on our observed depression in growth following treatment, we expected to see differences in the timing of treated versus untreated trout. Instead, treatment did not impact migration timing (i.e. stressed fish did not leave earlier or later to compensate for lower growth rates). Smolt status (resident or migratory) is largely determined in the fall and dependent on a trout's condition at that time (Metcalfe 1998). Therefore, despite the observed changes in length and mass following an early spring stressor (as seen in the recaptured individuals), all migratory trout still proceeded to exit the system at the same time. Furthermore, since there were no significant differences in terms of the number of trout recaptured from each treatment, it does not appear that an early spring stressor influences their ultimate smolt status.

While physiological status may determine whether a fish remains in a system or migrates out of the system (Nielsen et al. 2004, 2006), there is evidence that the actual timing of migration is more linked to environmental factors including photoperiod, temperature and water discharge (Hoar 1988, Aarestrup et al. 2002, Jonsson & Jonsson 2002). Synchronized migration (i.e. migrating en mass), which may occur during periods of high flow, helps to reduce predation by increasing turbidity in the water, decreasing the amount of time it takes to exit the system, and reducing the probability of predation for individuals within a larger group (Aarestrup et al. 2002). Therefore, while a springtime stressor may change the growth patterns of individual fish, they will still migrate at the same time as other, less stressed individuals. Consistent migration timing has important implications for sub-populations that experience greater stressors than other populations in their region. If all trout are migrating at approximately the same time, then trout that experience a stressor in their natal grounds will ultimately be less successful because they will undertake the migration in an inferior condition. While it has been shown that stressed individuals can compensate for early stressors and the resulting growth depression (Johnsson & Bohlin 2006), if there is not sufficient time for juvenile trout to compensate for this stressor (as in this study with an early spring stressor), they will likely be less successful in the ocean.

Survival

Migratory species are naturally exposed to higher levels of predation than their more resident counterparts (Gross et al. 1988), a pattern documented for brown trout (Elliott 1993). Despite the energetic advantages of migrating to the ocean, mortality rates can be double for trout that reach the ocean (0.25 % d^{-1} in the ocean, 0.12 % d^{-1} for resident fish; Elliott 1993). In addition, along the migratory pathway, mortality rates may also be quite high with documented mortality values ranging from 20 to 34 % within a few days of entering the estuary (Jepsen et al. 2006, Koed et al. 2006). In a natal stream, we observed significantly fewer HI fish passing both stations in comparison to CO fish. There was also a general trend towards fewer fish passing the arrays with increasing cortisol dosages. Because we observed no differences among treatments in the number of recaptures, the observed decrease in passage was likely the result of increased in-stream mortality during migration for the treatment groups. O'Connor et al. (2010) observed increased locomotor activity in cortisol-treated fish, and since increased activity is associated with an increased risk of predation (Jönsson et al. 1996), the observed decrease in survival of HI trout could be driven by increased activity. However, it should be noted that if increased activity by HI trout was the main driver in their reduced survival, we would also expect lower numbers of HI individuals among our recaptures, which was not observed. It is also possible that cortisol treatment affected immunocompetence (Carlson et al. 1993, Espelid et al. 1996) such that disease contributed to mortality; however, we did not evaluate post-treatment immune function or disease burden. It is therefore likely that compared to migratory CO trout, multiple compounding issues act to decrease survival of migratory HI trout including lower length- and mass-specific growth rates, decreased survival in-stream (whether due to predation or disease), and ultimately decreased survival upon reaching the ocean.

Techniques

PIT telemetry was a critical component of this study as it allowed us to tag and individually identify hundreds of fish rapidly, inexpensively, and with assumed minimal negative side effects (Ombredane et al. 1998, Gries & Letcher 2002, Zydlewski et al. 2006). This technology is ideally suited for application in stream systems where fish cannot avoid the antenna array. The paired use of antenna allowed us to determine the direction of fish movement and resulted in high levels of detection efficiency (>95 %) relative to previous studies (Zydlewski et al. 2006). The most important advantage of this technology is that the tags will continue to function indefinitely allowing us to monitor the return migration of trout back into the Gudsø Stream. Although we did not conduct a tag retention study, previous tag retention studies on juvenile salmonids reveal high (i.e. >99 %) retention of PIT tags such that tag loss is unlikely to be contributing to observed patterns (Gries & Letcher 2002).

While the pairing of PIT telemetry with cortisol manipulation enabled us to individually assess a trout's response to a stressor, there are some limitations regarding cortisol manipulations that should be acknowledged. When cortisol is released following a natural stressor, its concentration typically decreases following a peak at ~24 h due to a negative feedback loop (reviewed in Mommsen et al. 1999). The artificial increase in cortisol used in this study overrides this feedback and prolongs the stress response beyond a natural timeline. While this is a cause for concern, intracoelomic injection has been widely used (see Gamperl et al. 1994) since currently there are few alternatives that allow for the release of treated fish back into their natural setting. As such, the results presented in this study likely represent the extreme spectrum of possible outcomes for chronically stressed fishes. In addition, we did not measure cortisol titers in fish following injection. However, there have been numerous other studies that involve use of cortisol injection in a cocoa butter carrier on *Salmo trutta* (Pickering et al. 1982, Pickering 1989, Fevolden et al. 1991, Pottinger et al. 1992) such that there are well-established relationships between dose and subsequent level and duration of cortisol elevation. A surprising finding of this study was the apparent impact of the cocoa butter itself. Despite its previous successful use as a vector for cortisol for brown trout, we still observed significant impairment (relative to the CO group) in length-specific growth. Furthermore, despite a decreasing trend with the degree of treatment, the SH group was not statistically different from the cortisol-treated groups for length- and mass-specific growth, survival to S1, and survival to S2 (only for LW treatment). This would suggest that for wild trout, the intracoelomic injection of cocoa butter might act in a similar manner as an artificial elevation of endogenous cortisol.

In conclusion, using a combination of biotelemetry and a physiological manipulation we were able to track fitness-related endpoints of sea trout and show that a pre-migration experimental stressor increased in-stream mortality and impaired body condition. The long life span of PIT tags will enable us to continue to track fitness at an individual level and determine whether effects from a pre-migration stressor carryover as they grow and develop in the ocean and eventually return to their natal habitat to spawn.

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PAPER II

An experimental field evaluation of winter carryover effects in semi-anadromous brown trout (*Salmo trutta*)

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Caudal fin of a juvenile brown trout (*Salmo trutta*). Photo: Martin Hage Larsen.

An experimental field evaluation of winter carryover effects in semi-anadromous brown trout (*Salmo trutta*)

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Running headline: Winter carryover effects in brown trout

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Abstract

For semi-anadromous brown trout, the decision whether or not to smoltify and migrate to the sea is believed to be made at the end of the preceding summer in response to both local environmental conditions and individual physiological status. Stressors experienced during the fall may therefore influence their propensity to migrate as well as carry over into the winter resulting in mortality when fish face challenging environmental conditions. To evaluate this possibility, we artificially elevated cortisol levels in juvenile trout (via intracoelomic injection of cortisol in the fall) and used passive integrated transponder tags to compare their overwinter and spring survival, growth, and migration success relative to a control group. Results suggest that overwinter mortality is high for individuals in this population regardless of treatment. However, survival rates were 2.5 times lower for cortisol-treated fish and they experienced significantly greater loss in mass. In addition, less than half as many cortisol-treated individuals made it downstream to a stationary antenna over the winter and also during the spring migration compared to the control treatment. These results suggest that a fall stressor can reduce overwinter survival of juvenile brown trout, negatively impact growth of individuals that survive, and ultimately result in a reduction of migratory trout. Carryover effects such as those documented here reveal the cryptic manner in which natural and anthropogenic stressors can influence fish populations.

Key-words: glucocorticoid; stress; cortisol; winter; stream; passive integrated transponders; carryover effect

Introduction

Underlying life-history decisions and trade-offs in wild animals are complex physiological processes (Zera and Harshman, 2001) largely mediated by the endocrine system (Ricklefs and Wikelski, 2002; Crespi et al., 2013). As such, various challenges experienced by an animal (e.g., reductions in food availability, storms, predation events, and interactions with humans) that trigger a glucocorticoid stress response have the potential to alter life-history decisions and trade-offs through changes in energy allocation (Landys et al., 2006), potentially influencing reproduction and survival (Crespi et al., 2013). Migration associated with seasonal or life-history transitions represent a complex interaction between behaviour and physiology, which is particularly sensitive to various stressors (Dingle and Drake, 2007). Stress has the potential to have dramatic consequences on the decision of animals to migrate as well as their ultimate fate and fitness. Over the last decade, there has been growing recognition that carryover effects can occur where previous experiences appear to dictate the future state and survival of individuals (Harrison et al., 2011; O'Connor et al., 2014), often, but not exclusively, in the context of migration (Norris and Taylor, 2006) or seasonal transitions such as winter (O'Connor et al., 2010). These highly complicated interactions between organismal physiology, behaviour and life-history present experimental biologists with great opportunity to elucidate ecological and evolutionary processes, particularly in the face of changing environments (Wingfield, 2008).

Winter is an extremely challenging period for many animals in temperate regions because it is typically characterized by harsh environmental conditions and reductions in food availability. There are diverse strategies for dealing with winter challenges including migration (e.g., many birds) and hibernation (e.g., some mammals, reptiles or amphibians). However, some animals (e.g., fishes) remain active, although often somewhat quiescent compared to their behaviour in other seasons, and attempt to forage and survive in winter conditions (Shuter et al., 2012). Many juvenile fish species are particularly prone to overwinter mortality, with fish size and energetic condition influencing survival (Cunjak, '96; Hurst, 2007; Shuter et al., 2012). Fish in fluvial systems also have to deal with dynamic ice conditions (Brown et al., 2011). An added complication is the potential for external perturbations (stressors such as those induced by human activities) to further reduce the likelihood of survival during the winter. O'Connor et al. (2010) demonstrated increased overwinter mortality in largemouth bass (*Micropterus salmoides*) following exposure to an early season stressor (i.e., a carryover effect). For individuals that are able to survive winter conditions, these carryover effects can potentially resonate beyond a single winter, influencing other parts of their life history, and ultimately organismal fitness and population-level processes (Calow and Forbes, ‘98).

Brown trout (*Salmo trutta*) are native to almost all of Europe, but have been introduced widely across the entire globe (Jonsson and Jonsson, 2011). Populations in Denmark exhibit partial migration with a portion of the population in a system becoming resident and the remaining individuals migrating to the ocean (Alerstam et al., 2003). The decision to become a migrant or resident is driven by a variety of environmental and physiological variables including the availability of food, metabolic activity of the individual, and their condition (Thorpe et al., ‘92; Metcalfe, ‘98; Cucherousset et al., 2005; Boel et al., 2014), such that individuals with higher metabolic requirements and lower condition scores are more likely to undertake the migration to the ocean (Forseth et al., ‘99; Morinville and Rasmussen, 2003; Boel et al., 2014). The process of smoltification is inherently stressful and represents a trade-off between increased predation during migration and increased growth and reproductive potential for trout that forage in the marine environment (Hutchings and Myers, ‘85; Klemetsen et al., 2003). For anadromous brown trout populations, smoltification is thought to commence in the late summer and early fall of the preceding year when local conditions drive a portion of the population to begin a physiological transformation in preparation for the marine environment (Metcalfe, ‘98).

Given the importance of the fall season for smoltification and the fact that it precedes winter, juvenile trout at this time are likely sensitive to external perturbations that may result in sub-lethal stressors. Such stressors can be caused by natural (e.g., predation attempts, food scarcity) or human (e.g., point source pollution, habitat alterations) induced disruptions to the natural balance (Underwood, ‘89; Wingfield, 2003 Helmuth, 2009; Baker et al., 2013). Previous work on the influence of stress on wild anadromous trout suggests that, while an artificial stressor applied immediately prior to outmigration does not appear to influence the timing of migration, migration success is depressed relative to a control (Midwood et al., 2014). Similarly, growth of stressed individuals that remained in the stream (residents) was depressed. Over a longer timescale, there is therefore potential for a stressor applied in one season to influence the subsequent smoltification process and also result in carryover effects during the winter. Consequently, our goal was to evaluate the influence of a fall stressor on the overwinter survival, growth, and success of migration of juvenile brown trout. The potential confounding interaction between initial state (e.g., condition) and a fall stressor as well as the impact of initial state on migration status were also explored. Although carryover effects have been well-studied in migratory birds in terms of how conditions of overwintering grounds influence subsequent post-migration reproduction (e.g., Marra et al., ‘98; Norris, 2005), there is comparatively less known about how a stressor may influence life history trade-off decisions for species that must weigh the risks and benefits associated with migrating or becoming a resident. Indeed, there is a rich theoretical literature on how physiology (especially endocrinology and oxidative stress) mediates life-history trade-offs (see Zera and Harshman, 2001; Monaghan et al., 2009), yet experimental evaluations of such trade-offs in wild animals are lacking.

We use an experimental approach, comparing the survival, growth, and migration success between a control group and trout that received an artificial stressor (intracoelomic injection of cortisol). This experimental manipulation of GC levels via implantation of a GC-bearing vector is an increasingly common approach for the study of carryover effects in wild animals across a range of taxa (e.g., fish, Gamperl et al., ‘94; O’Connor et al., 2010; Midwood et al., 2014; birds, Spée et al., 2011; Davies et al., 2013; reptiles, Meylan et al., 2002; Cote et al., 2006). There are also numerous laboratory-based studies that manipulate GCs in fish (reviewed in Gamperl et al., ‘94) and provide a strong foundation for conducting field-based studies. Based on previous work with brown trout and other fishes, we predict that growth, migration success, and survival will be lower for fish with manipulated GCs relative to the control. We also predict that the initial state or condition of individual trout will dictate their predisposition to migrate or become a resident such that GC-manipulated individuals (with potentially impaired condition) will be more likely to migrate.

Methods

Study site description

Gudsø Stream is located in central-eastern Jutland, Denmark (Figure 1). Including a series of sub-tributaries, the stream flows ~16 km before entering into the northwestern Baltic Sea at Kolding Fjord. The stream supports a population of semi-anadromous brown trout comprised of both resident and migratory individuals; however, approximately two-thirds of the population migrates to the ocean, with more than half making this migration over a four-day period in 2013 (Midwood et al., 2014). Two PIT reading stations established approximately 1 km from the outflow of the stream into the fjord continuously log the passage of passive integrated transponder (PIT) tagged fish. Station 1 (S1) is located approximately 150 m upstream of Station 2 (S2). Detection efficiency was estimated for S1 as 93.9% after Zydlewski et al. (2006); it could not be estimated for S2 due to the absence of downstream detections. A small millpond located between S1 and S2 may potentially serve as a sink if individuals cannot find the outflow, therefore lower detection numbers are to be expected at S2. The millpond was not surveyed during the present study as water depth precluded access.

Capture, tagging and treatment

From November 4th to 7th, 2012, brown trout were collected from 5 sections in Gudsø Stream using single-pass backpack electroshocking (Scubla ELT 60 II G, running at 300 volts). Fish were immediately netted and placed into a container with regularly exchanged stream water. First, the total length (±1 mm) and wet mass (±0.1 g) of each fish were measured. A relative condition factor (K_R) was developed based on the relationship between log-transformed length and mass of the trout sampled in the current study (after Le Cren, '51). For each individual, K_R was then calculated using the following equation: $K_R = \log(\text{Mass})/(-2.03 + 3.03(\log(\text{Length}))$. Next, a 23 mm PIT tag (Texas Instruments, RI-TRP-RRHP, 134 kHz, 0.6 g mass in air, Plano, Texas, USA) was inserted into the body cavity of each fish as an individual marker. A recent evaluation of this technique with Atlantic salmon (*Salmo salar*) suggested that retention of the tags is very high (97%) with no mortality and no impacts on growth relative to a control group (Larsen et al., 2013). Tagged fish were then assigned into either control or cortisol treatments using a stratified random approach to ensure roughly equal sample sizes per treatment. Immediately following PIT tag insertion, cortisol fish received an intracoelomic injection of a suspension of cocoa butter (100% pure cocoa butter, Now Foods, Bloomingdale, IL) and hydrocortisone 21-hemisuccinate (Sigma-Aldrich, Product #H2882-1G) at a dosage of 100 mg kg^{-1} to raise circulating plasma cortisol levels to between 20-40 ng ml^{-1} for 2 to 4 weeks (Pickering, '89). When brown trout are exposed to acute stressors (e.g., variation in water flow or handling stressor; Pickering et al., '82; Flodmark et al., 2002), plasma cortisol typically rises to between 100-150 ng ml^{-1} but only stays elevated for several hours (Pickering et al., ‘89). Conversely, a chronic stressor (e.g., confinement or crowding) has prolonged elevation (~4 weeks) but lower magnitude (10 ng ml^{-1}; Pickering et al., ‘89). Therefore, the magnitude of cortisol elevation targeted in this study was on the upper end of ecologically relevant values and the extended duration was consistent with the notion of a chronic stress (Nagrodski et al., 2013). No sham treatments (receive injection of only cocoa butter) were used because, while there may be some minor negative impacts on growth, survival is not different than a control (Midwood et al., 2014). Fish from both treatments were allowed to recover for approximately 30 minutes in separate tanks of fresh stream water before being released back into the same section of the stream where they were captured. Animal care approval for this study falls under the Danish Animal Experiment Inspectorate (License Number: 2013-15-2934-00808).

To evaluate growth and survival of trout that remained in the stream over the winter (defined here as November 7th until February 28th), 6 areas were sampled between February 28th and March 19th, 2013 (Figure 1). This time window was selected as it is before the peak migration of trout in Gudsø Stream that typically occurs in mid-April (Midwood et al., 2014). All captured trout were scanned to determine if they had been PIT tagged. Total length and mass of recaptured individuals were measured; the mass of the PIT tag (0.6 g) was subtracted from the mass of each recaptured individual. These numbers were used to calculate the percent change in length, mass, and K_R relative to initial capture. Recaptured individuals were released back into the stream. Data were downloaded from the PIT stations in June 2013, after the aforementioned peak spring smolt run. It should be acknowledged that absolute survival was not evaluated, rather detection, both at the stationary antenna and during the spring electrofishing, was used as a surrogate for survival.

Statistical analysis

A Student’s t-test was used to determine whether the initial mean length, mass and K_R differed between the control and cortisol treatments. Based on their known activity, trout within each treatment were divided into three groups: down-stream migrants (detected passing S1 and/or S2; herein referred to as “migrants”), residents (recaptured instream during spring surveys, but not detected passing S1 or S2), and individuals with unknown fates (neither detected nor recaptured, possibly deceased, herein referred to as “unknown”). To determine whether initial state differed among trout that became migrant, resident, or unknown, an analysis of variance (ANOVA) was used to compare initial K_R among these groups. A post-hoc Tukey HSD was used to evaluate significant differences among groups.

To evaluate migration success, Fisher's Exact tests were conducted to compare the total proportion of trout in each treatment reaching S1 and S2 as well as the combined proportion moving past both stations in winter and spring. Similarly, a product-limit log-rank survival analysis was conducted to determine whether the number of detections at S1 differed between the control and cortisol treatments. A Fisher's Exact test was also used to determine whether more individuals from the control or cortisol treatments were recaptured during spring surveys. By combining individuals from the migrant and resident groups, we were able to estimate overall survival for each treatment, which was compared using a Fisher's Exact test. In terms of shifts in migration strategy, the proportion of trout migrating in the cortisol and control treatments was calculated as the number of trout passing the stations divided by the total number of individuals that were assumed to survive (includes both residents and migrants).

To evaluate the potential interaction between initial state (K_R) and treatment on survival, a logistic regression was performed. The survival response was split into known to survive (includes both migrants and residents, assigned a value of 1) and unknown (assigned value of 0). Finally, a Student's t-test was used to compare growth for length, mass, and K_R, measured as the percent change from their initial size to recapture. Recapture data for both migrant and resident trout were pooled for these analyses due to low sample sizes when analyzed separately. Similarly, we did not include initial capture location in the analysis due both to low samples sizes and previous work suggesting there were nor differences in migration timing or success (Midwood pers. comm.). All analyses were completed in JMP 9.0 (SAS). Trout that were detected at S2 were included in the analysis due to the potential for novel fish detections at this station; however, survival between the two stations was not evaluated since we were unable to evaluate the effectiveness of S2 and it was possible that it had lower detection efficiency than S1. Since diminished detection efficiency at S2 would have equally affected both control and cortisol tagged individuals a comparison between treatments of trout detected at S2 should still be valid.

Results

In total, 473 trout were captured, tagged and treated, 232 control fish and 241 cortisol-manipulated fish (Table 1). There were no significant differences in terms of initial length (t-test, $t_{(1)}$=-0.083, p=0.934), mass (t-test, $t_{(1)}$=-0.549, p=0.583) or K_R (t-test, $t_{(1)}$=0.0002, p= 0.999) between the two treatment groups. There were significant differences in initial K_R among the three trout groups (ANOVA; $F_{(2)}$=5.94, p=0.003), with K_R significantly lower in migrants relative to those with an unknown fate (mean±SD=0.99±0.02 and 1.00±0.03, respectively, Tukey-HSD; p=0.007); residents had an intermediate K_R that was not distinct from the other groups (mean=0.99±0.02). Given the small absolute difference among treatments, the ecological relevance of this finding is explored in the discussion.

There appears to have been substantial overwinter mortality for both the control and cortisol-treated fish, with 64% and 86% (respectively) of trout unaccounted for either by detection at S1 or S2 or during surveys in the spring (Table 1). Despite overall high mortality, significantly fewer trout in the cortisol treatment were detected or recaptured relative to the control (Fisher's Exact Test, $\chi^2_{(1)}$=39.13, p<0.0001). Trout were observed passing S1 and S2 both during the winter (Novbember 7th until February 28th) and in the spring (March 1st until May 30th); however, 80% of all cortisol trout that passed S1 did so in the winter, significantly more than the control (49%; Fisher's Exact Test, $\chi^2_{(1)}$=4.88, p=0.027). Similarly, based on the survival analysis, there was significantly lower survival of cortisol-treated trout at S1 than control trout ($\chi^2_{(1)}$=13.56, p=0.0002; Figure 2). Regardless of timing, only a small percentage of the control treatment migrated successfully past S1 and S2 (≤20% at both stations); however, this still represented significantly greater passage than the cortisol treatment (<10% at both stations; Fisher's Exact Test, S1, $\chi^2_{(1)}$=13.91, p=0.0002; S2, $\chi^2_{(1)}$=7.32, p=0.0068; Table 1). Similarly, based solely on resident trout, trout from the cortisol treatment were also significantly less likely to be recaptured during the spring surveys (Fisher's Exact Test, $\chi^2_{(1)}$=16.22, p<0.0001; Table 1) than control trout, suggesting again that overall there was lower survival among cortisol-treated fish. There was no clear shift in migration strategy (e.g., resident or migrant) for trout that were known to survive with relatively equal proportions of control (0.52) and cortisol-treated (0.55) trout passing the stations.

There was no interaction detected between K_R and treatment in determining the ultimate fate of a trout (Wald $\chi^2_{(1)}$=0.1, p=0.793). However, both individual terms were significant

(K_R, Wald $\chi^2_{(1)}$=11.9, p=0.001; Treatment, Wald $\chi^2_{(1)}$=30.3, p <0.0001) with greater survival for trout in lower initial condition and trout in the control treatment.

Due to the low number of cortisol-treated trout recaptured that became migrants (N=3), data from both migrant and resident groups were pooled to compare differences in growth between treatments. Over the 112 to 130 days from tagging to recapture, control trout grew significantly more both in terms of length (t_{58}=-5.779, p<0.0001; Figure 3) and mass (t_{58}=-4.951, p<0.0001; Figure 3) than cortisol-treated trout. In fact, rather than exhibiting growth, cortisol-treated trout on average lost 5.6% of their body mass compared to a 6.8% gain for trout in the control group (Figure 3). There was no difference in the percent change in K_R between the two treatments (t_{58}=-0.338, p=0.563; Figure 3).

Discussion

This study represents a simple experimental test of winter carryover effects in juvenile salmonids, with particular focus on how a fall stressor can have resulting impacts on survival, growth and down-stream migration success. Previous work has documented carryover effects in long-distance migrants (e.g., American redstarts, Norris et al., 2004; Black-tailed godwit, Gunnarsson et al., 2005; Light-bellied Brent goose, Inger et al., 2010; Sockeye salmon, Donaldson et al., 2010), which, in addition to exhibiting carryover effects in the winter (e.g., O'Connor et al., 2010), may be exposed to numerous and varied stressors during their migration. Our study is one of the first to integrate the concepts of carryover effects, winter ecology, and migration and evaluate their influence on the life history decision to migrate or remain resident. For cortisol-treated fish, the majority of the response metrics (e.g., growth, down-stream migration success, and survival) were depressed relative to fish in a control group, suggesting that impairments to pre-winter condition in brown trout influence individual overwinter survival and migration success. However, we found no evidence to support the prediction that elevated GCs influenced the determination of a migratory or resident life history strategy. Despite a strong theoretical basis for physiological status mediating life-history decisions (Zera and Harshman, 2001), our experimental approach failed to document such a relationship, at least in the context of elevated GCs, in a wild fish.

Overwinter survival

While the ultimate fate of each individual cannot be known for certain, since the majority of trout were neither detected at the stations nor recaptured in the stream, we conclude that there is naturally high overwinter mortality in the Gudsø Stream system for juvenile brown trout. Previous work has found a high-degree of variability in natural overwinter mortality of brown trout, ranging from 16% to 65% (Elliot, '93 reviewed in Huusko et al., 2007) and our values for control trout with unknown fates are at the upper end of this range. Similar to O'Connor et al. (2010), we found that a pre-winter stressor (injection of cortisol) significantly impacted survival with just over one third as many cortisol treated trout surviving relative to control. This increased mortality emphasizes the challenges faced by fish during the winter and shows how pre-winter condition may play a role in dictating their ability to survive the winter. We caution, however, that since we did not assess the timing of mortality in cortisol treated trout, it is possible that they experienced increased mortality shortly after treatment. A more detailed evaluation of post-treatment survival is therefore warranted. Regardless, the scale of winter mortality is of a magnitude that warrants attention of resource managers to ensure it is being appropriately incorporated into population models. For example, survival is much lower during winter than the initial marine mortality of smolts that is normally hypothesized to be a critical life stage (Thorstad et al., 2012; Aarestrup et al., 2014; del Villar-Guerra, 2014).

While there were significant differences in the initial condition of individuals in the migrant and unknown groups, the absolute difference in condition was only 0.01. As a result it is likely that this difference is not ecologically relevant. Furthermore, this initial state was not found to interact with the stress treatment, suggesting that the treatment had an impact regardless of the initial state of the individual. Storage of lipids prior to the winter is an important part of the overwinter survival strategy of many animals (e.g., salmonids, Berg and Bremset, '98; reptiles, Derickson, '76; birds, Blem, '76). Prolonged activation of the glucocorticoid pathway, and the resulting increase in metabolic activity, can prevent the creation of these reserves or deplete established stores (Sheridan, '86; reviewed in Mommsen et al., '99). This loss of lipid reserves is evident in the cortisol trout that survived the winter given their lower mass and overall limited growth. Since low lipid reserves prior to winter leads to a decrease in survival (e.g., rainbow trout, Biro et al., 2004), it is probable that a decrease in lipid reserves in the cortisol treatment resulted in lower survival of these individuals. In addition, as discussed in Midwood et al. (2014), it is also possible there are differences in predation rates between the two treatments resulting from differences in behaviour or energetic ability. However, to our knowledge, there is as yet no evidence for increased predation of stressed individuals. Finally, it is also conceivable that GC manipulation promoted foraging and thus energy accumulation through time, although that is not something that we were able to find support for in the literature. Because we were unable to sample fish repeatedly, it is not possible to identify the specific manner in which GC manipulations were manifested which limits our ability to elucidate how GCs, overwinter survival and migration success are linked.

Migration timing and strategy

For trout that were known to survive, roughly equal proportions of control and cortisol trout passed the stations. This suggests that once an individual made the decision to migrate downstream at the end of the summer or early fall, a subsequent stressor did not appear to alter their migratory or resident life history strategy. However, given low samples sizes in both treatments due to high overwinter mortality, this is not conclusive evidence of an absence of an effect of stress on the propensity to migrate and this hypothesis should be explored further.

Typically, migration to the ocean occurs during the spring, therefore it was surprising to find trout moving downstream in the winter. Indeed, 80% of all migratory trout in the cortisol treatment and 49% of control trout moved downstream past the stations in the winter. An important caveat to note regarding this observation is that there is still approximately 1 km of stream habitat downstream of the stations; therefore, it cannot be conclusively stated that the movements observed during the winter were made by ocean-bound migrants or that they actually entered saline waters during winter. These winter migrants may instead reflect a portion of the population that spends the winter in the section of Gudsø downstream of the stations. That none of these individuals was observed moving back upstream, as might be expected if they were using this solely as overwintering habitat, suggests that they 1) did not survive, 2) migrated to the ocean, or 3) became residents downstream. Positioning of detection antenna closer to the estuary, analysis of return data, or electroshocking downstream of the PIT antenna could help resolve the ultimate destination of these types of individuals.

Overwinter change in mass

Growth depression following a stressor is a common response that has been observed in birds (e.g., Barn owls Almasi et al., 2013; Abert’s towhees Davies et al., 2013), fishes (e.g., Largemouth bass O’Connor et al., 2011), reptiles (e.g., Common lizard, Cote et al., 2006), and mammals (e.g., Belding’s ground squirrel, Brooks and Mateo, 2013). This impairment is driven by increased metabolic rate and the resulting loss of energy reserves (Chan and Woo, ‘78; Morgan and Iwama, ‘96; O’Connor et al., 2011); indeed the cortisol-treated fish in this study lost over 5% of their mass over the winter while the control treatment gained over 6%. While these types of losses can be compensated for in a comparatively short time period (e.g, 1 month), there is potential for this compensation to have a carryover effect in the future life of these individuals resulting in lower survival in the ocean (Johnsson and Bohlin, 2006). Thus, even if a stressed individual survived the winter, their long-term survival may still be impaired. This type of long-term carryover effect can be challenging to document, but is critical to evaluating the complete impact of short-term stressors.

Previous work has also suggested that brown trout may experience growth depression over the winter in the absence of a stressor due to higher energetic costs associated with maintaining metabolic activity (Cunjak and Power, ‘87; Berg and Bremset, ‘98). Our results suggest that brown trout in Danish streams continue to grow throughout the winter in the absence of a stressor (as witnessed by the increased mass in the control treatment). This discrepancy may be related to the severity of the winter wherein ice may form over streams in some regions. In Gudsø Stream, water temperatures typically remained well above 1°C (mean = 3.8±2.0 °C) in the winter (unpublished data not shown,).

Caveats

An important caveat of the work presented here is that the ultimate fate of individuals not detected at the stations or recaptured in the stream cannot be known for certain. That being said, a comparison of the proportion of individuals in the two treatments detected passing the stations and recaptured in the streams likely represents a valid comparison since there is no reason to believe that the probability of detection in either of these two instances is dependent on treatment. Detection at PIT stations is more dependent on the orientation of the tag and the strength of the electromagnetic field (Burnett et al., 2013); therefore, the detection (or missed detection) rate would likely be similar for the two treatment groups. Capture of brown trout using electrofishing is dependent on size, but typically is quite efficient since they are actively drawn to the anode (efficiency range 52-90%; Büttiker, ‘92); this, in combination with a narrow stream (typically less than 2 m) and shallow water depths (<0.5m), likely resulted in high capture efficiency in sampled reaches. Our recapture efforts in the spring of 2013 covered a larger area than the initial fall surveys (21.6% of Gudsø Stream versus 10.7%, respectively) but, without sampling all areas of the stream (> 16 km), we cannot conclusively determine the proportion of the population that survived the winter. However, efforts were likely sufficient to provide a comparison of cortisol versus control treatments since we sampled throughout the stream in areas that likely provide good habitat (e.g., overhanging cover, instream structure) and also resampled areas that were part of the initial fall surveys.

Finally, we must caution some of the interpretations of the growth results, especially as they relate to the cortisol treatment. Without a sham treatment, we cannot directly differentiate between the impact of exogenous cortisol manipulation and the cocoa butter vector. As previously mentioned, a sham treatment of cocoa butter did influence the growth, but not survival, of wild brown trout (Midwood et al., 2014). There was still, however, a trend towards lower growth rates for the cortisol treated individuals relative to the sham treatment. Therefore, in the current study, we cannot be certain of the portions of the change in growth that can be attributed to the cortisol treatment and the cocoa butter vector. Both the current study and Midwood et al. (2014) had low recapture rates (~5-10%) largely due to the timing of recapture efforts post-migration. A study of wild trout during migration would result in larger sample sizes that would help to partition the impacts of the cortisol treatment and the cocoa butter vector.

Conclusion

Carryover effects have the potential to be the main drivers behind variability in individual behaviour, reproduction, and ultimately, survival (Harrison et al., 2011). A more complete understanding of their potential influence on survival, growth, migration success, and selection of a life history strategy (e.g., to migrate or become resident) will help determine the drivers behind population dynamics. This study used a simple experimental approach to document the negative impacts to growth and survival of an overwinter carryover effect following a late fall stressor. Notwithstanding the aforementioned caveats, it is evident from this and other studies that winter is a challenging period for juveniles and that a preceding stressor can have a later impact on the survival of affected individuals. It is therefore important for environmental managers and researchers to recognize that impacts from a stressor applied during one season may not manifest themselves until later in the life history of an individual. We must be aware of the potential influence of experience on the current behaviour, condition, and ultimately survival of each individual which is relevant to conservation and management of wild animals in a changing world (Wingfield, 2008; O'Connor and Cooke, 2015).

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Table 1. Summary of the number of brown trout tagged and the mean initial length, mass, and condition factor for each treatment with standard deviations. Migration success to S1 and S2 as well as the proportion of movements that occurred at these stations in the winter (November-February) and spring (March-May) are presented as a percentage and absolute number (in brackets. The percent and absolute number (in brackets) of trout from the treatments that were grouped as migrants, residents, or unknown are also shown. Finally, the number of individuals recaptured in the spring and the overall survival (including data from individuals that moved past S1 and S2 as well as individuals that were recaptured in spring 2013) are presented. Subscript letters indicate significant differences between treatments.

	Treatment	
Metric	Control	Cortisol
Sample Size	232	241
Length (cm)	14.1±2.1	14.1±1.7
Mass (g)	30.8±20.5	29.9±14.0
Relative Condition Factor (K_R)	1.00±0.03	1.00±0.03
% Reaching S1	20.3_A (47)	8.3_B (20)
% Reaching S2	14.2_A (33)	6.6_B (16)
% Migrants	20.3 (47)	8.3 (20)
% Residents	15.5 (36)	5.8 (14)
% Unknown	64.2 (149)	85.9 (207)
Spring Recaps	44_A	16_B
% Overall Survival	36.0_A (83)	14.1_B (34)

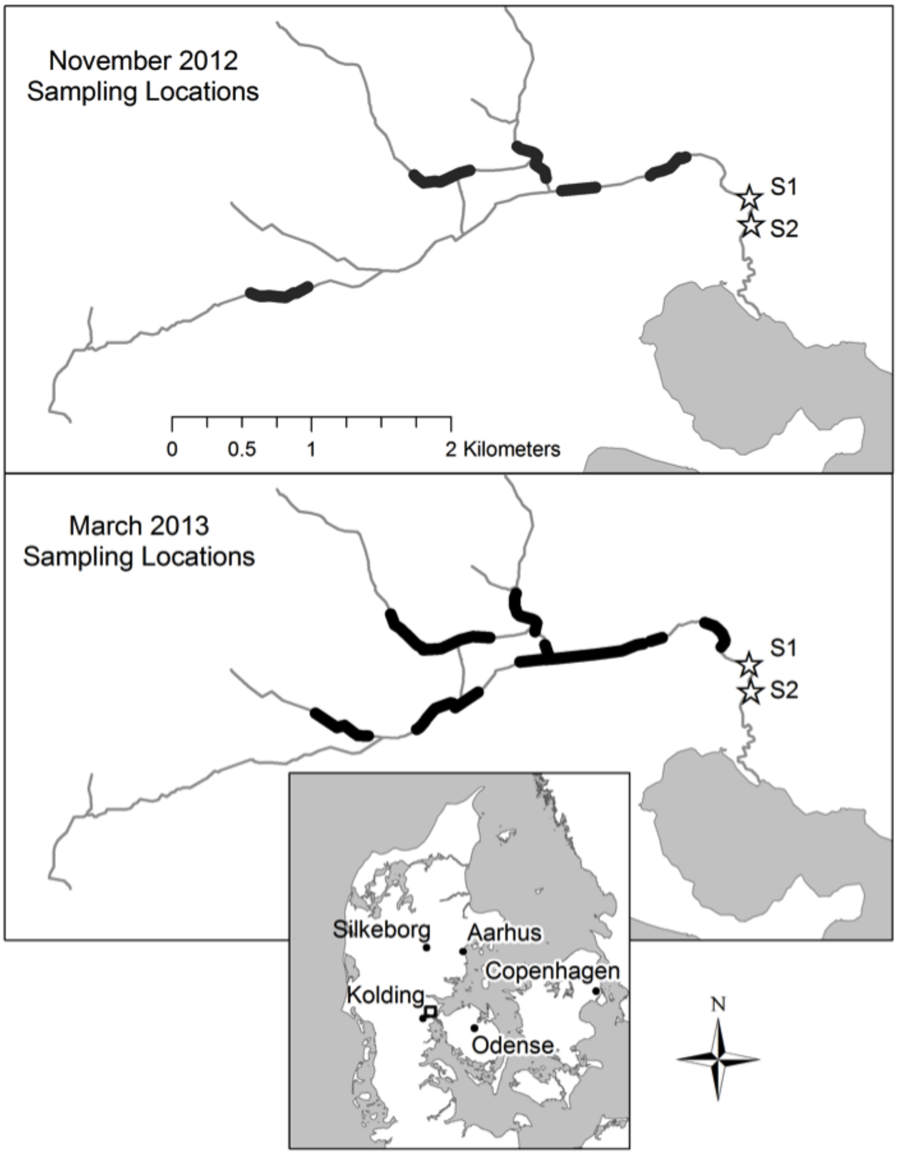

Figure 1. Location of Gudsø Stream and position of the two PIT antennas, S1 and S2. Sampling locations for initial capture (November 2012) as well as areas re-sampled in the spring of 2013 are shown as darker lines. Light grey areas represent water and the outflow location of Gudsø Stream into Kolding Fjord. Bottom inset shows locations of Gudsø Stream north-east of Kolding.

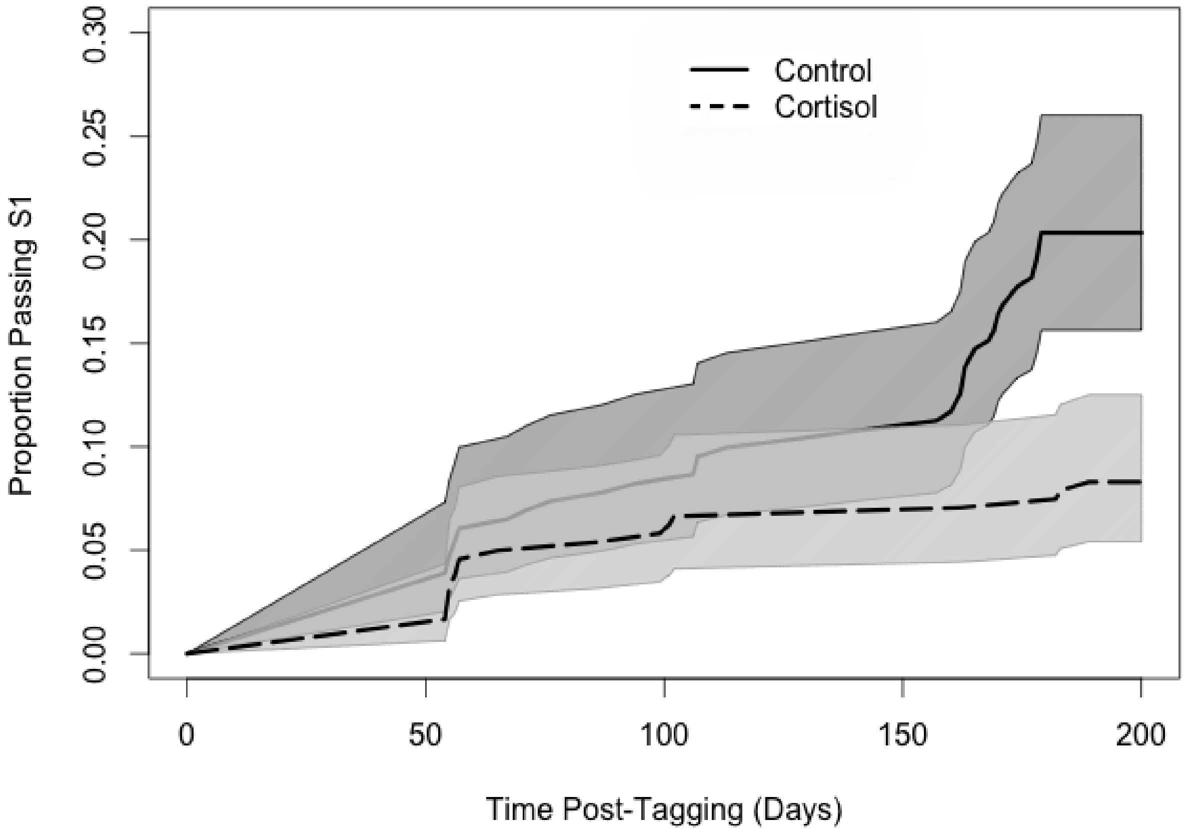

Figure 2. Visualization of the output from the product-limit log-rank survival analysis. Trout from the cortisol treatment had significantly lower survival to S1 than control trout. Shaded areas show the 95% confidence intervals.

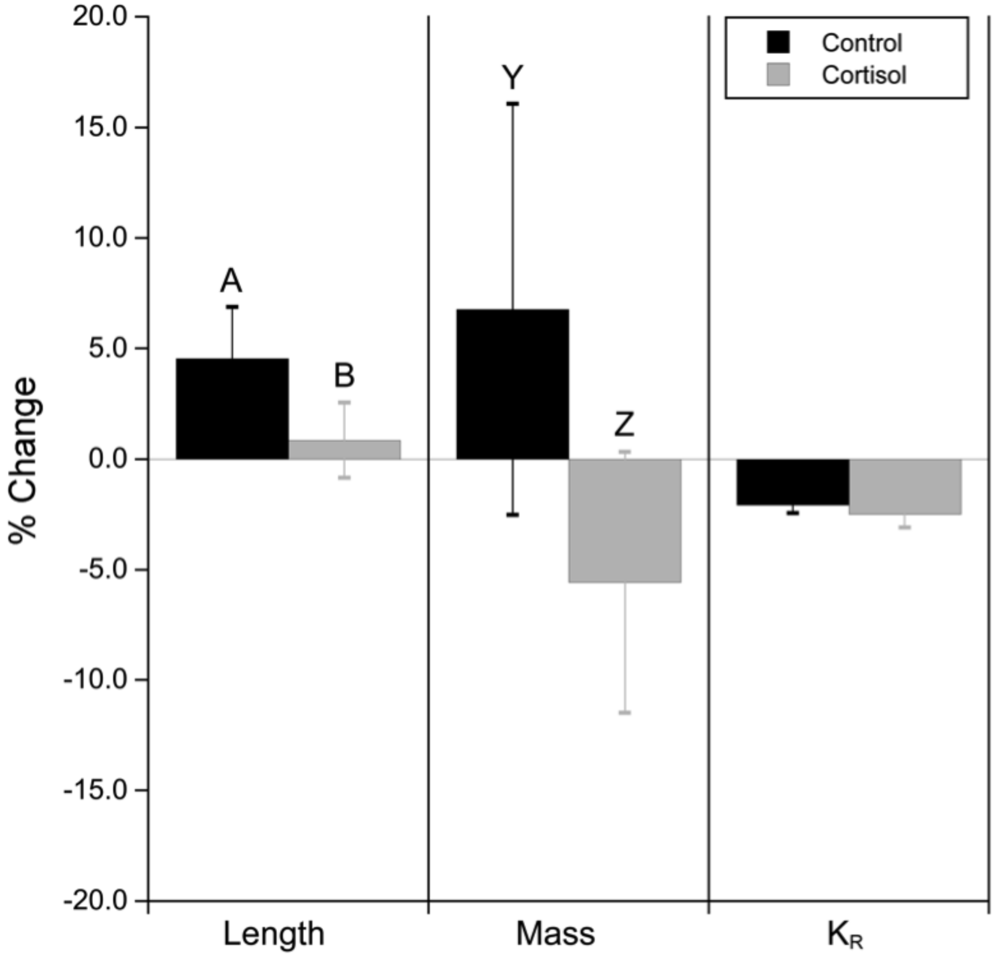

Figure 3. Percent change in length, mass, relative condition (K_R) for trout recaptured in the spring of 2013 prior to the main downstream migration. Letters indicate significant differences. The error bars show 95% confidence intervals.

PAPER III

Reduced rearing density increases post-release migration success of Atlantic salmon (*Salmo salar*) smolts

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Hatchery-reared Atlantic salmon (*Salmo salar*) parr and smolts. Photo: Martin Hage Larsen.

Reduced rearing density increases post-release migration success of Atlantic salmon (*Salmo salar*) smolts

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Abstract

The overall aim of this study was to investigate the effect of rearing density on the post-release survival of Atlantic salmon (*Salmo salar*) smolts during seaward migration. Fish were either reared at conventional hatchery density or at one-third of conventional density. Three hundred one-year old smolts from each density treatment were individually tagged with passive integrated transponder (PIT) tags and released 3.2 km upstream of a stationary antenna array in a natural stream. There were no significant differences in length, body mass, or condition between fish from the two density treatments during rearing in the hatchery. However, individuals reared at reduced density had less eroded dorsal fins and opercula relative to those from the high-density treatment. In the stream, the downstream migration success was 16% higher for fish reared at reduced density than for conspecifics kept at high-density, but the timing of migration was similar for both groups. These novel results suggest that conventionally high rearing densities may reduce welfare and the post-release migration and survival of hatchery-reared Atlantic salmon.

Key-words: stock enhancement; fisheries management; telemetry; fin erosion; salmonids; welfare

Introduction

Hatchery supplementation programs are widely implemented to mitigate losses in declining or threatened populations of salmonids from anthropogenic factors, such as habitat degradation, damming of rivers, pollution, and overexploitation (Parrish et al. 1998; Fraser 2008; Jonsson and Jonsson 2009). However, because the post-release survival of hatchery-reared salmonids is often considerably lower than that of their wild counterparts (e.g., Jonsson et al. 2003; Jonsson and Jonsson 2006; Aarestrup et al. 2014), the efficiency of such hatchery programs has been debated (Brannon et al. 2004). Saloniemi et al. (2004), for example, showed that wild Atlantic salmon (*Salmo salar*) had a 4.5 times higher smolt-to-adult survival rate than cultured fish in a northern Baltic river. Changes in behavioural, physiological, and morphological phenotypes due to the artificial rearing environment have been recognized as major factors contributing to the poor survival of hatchery-reared fish in nature (Brannon et al. 2004; Huntingford 2004; Fraser 2008).

Several studies have evaluated the potential for improving post-release performance of hatchery-reared salmonids by using a variety of different rearing methods (Brown and Laland 2001; Brown and Day 2002; Näslund and Johnsson in press), but surprisingly few have tested and verified whether these methods are applicable on a larger scale to increase survival of released fish in nature (but see Brockmark and Johnsson 2010; Hyvärinen and Rodewald 2013; Kavanagh and Olson 2014). Rearing at reduced hatchery density has recently shown promising results in increasing post-release survival of hatchery-reared salmonids (Brockmark and Johnsson 2010; Barnes et al. 2013; Kavanagh and Olson 2014). For instance, studies on brown trout (*Salmo trutta*) parr demonstrated that reduced rearing density facilitated development of adaptive behaviour and resulted in increased survival and growth following release into their natural stream environment (Brockmark and Johnsson 2010; Brockmark et al. 2010). Similar results have been obtained for Pacific steelhead (*Oncorhynchus mykiss*), where reduced rearing density increased the smolt-to-adult survival rate (Kavanagh and Olson 2014). Studies on Pacific salmon, however, have produced variable effects of rearing density on post-release survival, and are probably a reflection of variation in rearing facilities (e.g., ponds vs. raceways), production intensity, species tolerance to crowding, and natural conditions (Ewing and Ewing 1995; Barnes et al. 2013; Clarke et al. 2013).

Clearly there is a need for additional research focused on the effects of hatchery rearing density on post-release performance of salmonids.

Atlantic salmon for supplementary purposes are often released into rivers as smolts in spring, shortly before or at the time of seaward migration of their wild conspecifics (Jonsson and Jonsson 2009). Stocked fish have little time for adapting to the novel natural environment before migration should start, and therefore, any maladaptive traits may lead to mortalities at this stage (Jonsson and Jonsson 2006). Given that the mortality at the smolt stage contribute to determine the abundance of adult spawner returns, it is imperative to understand the potential effects of rearing density on behaviour and survival of stocked smolts during migration to the sea. However, to our knowledge, no studies have thoroughly evaluated the effect of rearing density on post-release performance of Atlantic salmon (see Brockmark et al. 2007).

The objective of this study was to compare the seaward migration success of one-year old Atlantic salmon smolts reared at conventional hatchery density and one-third of conventional density. Fish were released during spring in a natural stream and their decent was monitored using passive integrated transponder (PIT) telemetry. Based on previous work with salmonids discussed above, we hypothesize that fish reared at reduced density would be more successful of undertaking the migration to the sea than individuals from a high-density environment.

Materials and methods

Experimental fish and rearing conditions

The fish used in this study were first-generation hatchery offspring (F1) of Atlantic salmon originating from River Storå, Denmark. The parental fish were caught in late autumn 2011 by electrofishing and transported to the Danish Centre for Wild Salmon (DCV) in Randers. The eggs from 47 females were stripped and fertilized by mixing with milt from 27 males. Fertilized eggs were incubated in egg trays and hatched in late March 2012. After yolk sac absorption, experimental fish were transferred to indoor conventional hatchery tanks (200 × 200 cm) for exogenous feeding at two rearing densities: high density (HD) and low density (LD). The HD treatment was set according to the local hatchery practice and the LD treatment was set to one-third of standard density. The initial number of fish in each tank was 30 000 (7 500 fish m^{-2}) for the HD treatment and 10 000 (2 500 fish m^{-2}) for the LD treatment. On 16 July 2012, as fish grew, the number of fish was haphazardly lowered to 6 000 (1 500 fish m^{-2})

and 2 000 (500 fish m^{-2}) individuals per tank in the HD and LD treatment group, respectively. Each density treatment was replicated in three hatchery tanks. Water depth in the tanks was kept at 35 cm, resulting in a water volume of 1 400 L. The tanks were individually supplied with water from a recirculation system at a flow rate of 30 L min^{-1}. Throughout the rearing period, the average water temperature was 8.5 °C (range: 4.0–15.5 °C) and the indoor light regime followed the natural photoperiod cycle. The fish were fed with commercial trout pellets (Aller Performa, Aller Aqua, Denmark) equivalent to 2% of body mass per day using automatic feeders. The pellet size was changed in accordance to the manufacturer's recommendations as the fish increased in size.

Sampling protocol

Fish were sampled at three occasions during the rearing period: 12 July 2012 (n = 600), 5 November 2012 (n = 300), and 20 March 2013 (n = 300). At each sampling, equal numbers of fish were haphazardly collected from each replicate tank. Fish were anaesthetized with benzocaine (20 mg L^{-1}) and individually measured for total length (± 1.0 mm) and body mass (± 0.1 g). In addition, during the final sampling event on 20 March 2013, erosion to the fins (dorsal, pectoral, and caudal) and opercula was visually quantified using an ordinal scale of 0, 1, 2, and 3, corresponding to no erosion (0% eroded), mild erosion (1-24% eroded), moderate erosion (25-49% eroded), and severe erosion (≥50% eroded), respectively (after Hoyle et al. 2007). Fin and opercula erosion were measured as important indicators of aggression and stress levels during rearing (Ellis et al. 2002; Latremouille 2003; Huntingford et al. 2006). Fish were also examined for other external lesions on their bodies as evidence of aggressive encounters with conspecifics. All fins and opercula were scored by the same observer to avoid bias. Fish were returned to their respective tanks after these procedures.

Released fish

On 22 March 2013, 100 Atlantic salmon smolts ≥ 130 mm in total length were haphazardly sampled from each of the six hatchery tanks for release in a nearby natural stream (i.e., 300 fish from each density treatment). First, fish were anesthetized and measured for length and body mass as above. Next, fish were individually tagged by surgically implanting a 23 mm PIT tag (RI-TRP-RRHP, half duplex, 134 kHz, diameter 3.85 mm and weight 0.6 g in air; Texas Instruments, Plano, Texas, USA) into the peritoneal cavity through a small ventrolateral incision made slightly anterior to the pelvic fins (see Larsen et al. 2013). Following tagging, smolts were kept in a tank for observation during 12 days. There were no mortalities or tag loss during this period.

On 3 April 2013, the tagged fish were transported in two oxygenated tanks (each 750 L) to Gudsø Stream located in east-central Jutland, Denmark. The total transportation time was 1.5 hours and all fish were in good condition and showed no signs of abnormal behaviour. Atlantic salmon are not indigenous in Gudsø Stream, but it supports a wild population of anadromous brown trout. The fish were released on a 150 m stretch of the stream, 3.2 km upstream of a PIT antenna array consisting of two swim-through antennas spaced ~5 m apart. The antenna array was installed 1.1 km upstream from the stream outlet to Kolding Fjord (see Midwood et al. 2014 for a map of the study area). The antennae were operating continuously throughout the study period and the tag detection probability was estimated as 99.7% based on the methods described in Zydlewski et al. (2006). This setup allowed us to efficiently monitor the downstream movements of individual PIT-tagged Atlantic salmon smolts. On 18 and 19 June 2013, after the smolt migration had finished, the stream was surveyed by electrofishing from the antenna array to approximately 1.5 km upstream of the release site. Selected transects of all sub-tributaries that flow into the main stem of the stream were also sampled. Handling, rearing, and tagging of fish were conducted in accordance to the guidelines described in permission (2012-DY-2934-00007) from the Danish Experimental Animal Committee.

Statistical analysis

Linear mixed effects models (LMMs) were used to analyze effects of rearing density on length, body mass, and Fulton's condition factor (K = [100 × body mass (g)] / [length (cm)^3]) for the three sampling occasions in the hatchery. The model used was as follows:

(1) Response variable = Intercept + Treatment + Tank(Treatment)

with Treatment (LD and HD) as a fixed factor and replicate Tank nested within Treatment as a random effect variable. The model was fitted using restricted maximum likelihood (REML). We used the same model to compare length and Fulton's condition factor of released smolts among density treatments. Differences in intensity of fin and opercula erosion between density treatments were assessed using generalized linear models (GLMs) with a multinomial probability distribution and cumulative logit link function according to model 1, but with Tank(Treatment) as a fixed factor to detect possible effects of specific tanks. Likelihood ratio tests (LRT) were used to evaluate overall model significance. Separate analyses on fin and opercula condition were also performed for fish ≥ 130 mm in total length, corresponding to the size range of fish that were released into Gudsø Stream. Fin and opercula erosion was not quantified for the released smolts to minimize handling time.

Migration success in the stream was defined as a detection of an individual at an antenna. To analyze the effect of rearing density and fish length at time of release on the probability of successful migration we used a generalized linear mixed model (GLMM) with a binomial error structure and logit link function according to the following model:

$$\text{(2) Migration success} = \text{Intercept} + \text{Treatment} + \text{Length} + (\text{Treatment} \times \text{Length}) + \text{Tank(Treatment)}$$

with Treatment as a fixed factor, Length as a continuous covariate, and replicate Tank nested within Treatment as a random effect variable. A two sample Kolmogorov-Smirnov (K–S) test was used to evaluate the influence of rearing density on migratory timing, which was calculated as the number of days following release a fish was first recorded on a stream antenna.

All statistical analyses were performed in R 3.0.1 (R Development Core Team 2013) and SPSS 20.0 (IBM Corporation, Armonk, NY, USA). Prior to the analyses, data explorations were applied following a protocol described by Zuur et al. (2010). To determine minimum adequate models, we used backwards stepwise elimination of non-significant predictors with a retention threshold of $P < 0.05$. Assumptions of homogeneity of variance and normality were assessed by graphical inspection of the residuals. Independence was verified by plotting residuals versus each explanatory variable. Length and body mass data were log-transformed prior to analyses. Variation in association with recorded mean values is given as standard deviation throughout. Statistical significance for all analyses was set at $P < 0.05$.

Results

Effect of treatment during rearing

The average length, body mass, and Fulton’s condition factor did not differ between fish in the two density treatments at any of the three sampling occasions (LMM, $F \leq 1.914$, df = 1, $P \geq 0.239$; Table 1). However, fish reared at high-density had higher frequency and more severe dorsal fin and opercula erosion than those reared at reduced density (GLM, LRT ≥ 4.871, df = 1, $P \leq 0.027$; Table 2). We observed no damage to the caudal and pectoral fins in either of the two density treatments. Analyses on fish ≥ 130 mm in total length showed similar results, except that erosion to the right operculum could not be distinguished statistically among density treatment (GLM, LRT = 1.988, df = 1, P = 0.159). There were no significant differences in the level of fin and opercula erosion among replicate tanks within each treatment (GLM, LRT ≤ 9.429, df = 4, $P \geq 0.051$).

Downstream smolt migration

A total of 359 out of 600 released smolts were detected at the antenna array in Gudsø Stream, giving an overall migration rate of 60%. The downstream migration success was significantly higher (16%) for Atlantic salmon reared at reduced density than for individuals reared at high-density (GLMM, LRT = 4.517, df = 1, P = 0.034; Fig.1). The average length of released smolts differed between density treatments, with LD fish being slightly larger than HD individuals (LMM, F = 24.300, df = 1, P = 0.008; Table 1). However, length as well as the interaction between treatment and length had no significant effect on the probability of successful migration, and were thus excluded from the final model (GLMM, Length: LRT = 0.171, df = 1, P = 0.679; Treatment × Length: LRT = 0.418, df = 1, P = 0.518). There was no significant difference in Fulton’s condition factor between density treatments (LMM, F = 1.613, df = 1, P = 0.273; Table 1).

Atlantic salmon smolts were detected moving downstream from 3 April until 9 May 2013 (Fig. 1). The migratory timing did not vary significantly between density treatments, although smolts reared at high-density, on average, were delayed by four days (K–S test; Z = 1.279, P = 0.076; Fig. 1). None of the released fish were caught during subsequent electrofishing in the stream that occurred on 18 and 19 June 2013.

Discussion

The present results clearly demonstrate that lowering conventional rearing density can improve post-release survival of Atlantic salmon smolts during riverine migration to the sea. This finding is in good agreement with results obtained for other salmonid species, where reduced rearing density usually have been shown to increase survival of fish after stocking into nature (e.g., Brockmark and Johnsson 2010; Barnes et al. 2013; Kavanagh and Olson 2014). It is important to keep in mind that none of the released smolts were caught during the electrofishing surveys that occurred after the smolt migration had finished, indicating that individuals not detected at the PIT antenna station had died.

Body size is often a key determinant influencing survival of hatchery-reared Atlantic salmon smolts when released into nature (Hansen and Jonsson 1989; Saloniemi et al. 2004). While a majority of studies have found negative effects of increased rearing density on growth of salmonids (e.g., Refstie and Kittelsen 1976; Ellis et al. 2002; Brockmark et al. 2007), some argue that rearing density has no or little effect on growth as long as water quality is maintained at adequate levels and food ratios are within the recommended standards (Soderberg et al. 1987; Hosfeld et al. 2009). In this study, rearing density had no significant effect on growth and condition of fish based on the results obtained from the three sampling occasions in the hatchery. However, the average length of smolts released into the stream differed significantly between treatments, with fish from the high-density being slightly smaller (147.3 ± 7.12 mm) than individuals reared at low-density (152.2 ± 8.05 mm), suggesting that reduced rearing density produced larger smolts. The small difference in average length among density treatments, however, was not responsible for the noted difference in migration success in the stream.

Reduced rearing density has shown potential to promote development of important behavioural life skills in salmonids, such as foraging abilities and anti-predator response, resulting in increased growth and survival when released into nature (Brockmark and Johnsson 2010; Brockmark et al. 2010). At reduced densities fish are more able to swim around freely which may facilitate individual decision-making (Johnsson et al. 2014), whereas increased densities may promote schooling and/or result in sensory overload at the cost of reduced ability to learn individual skills (Dukas 2002; Laland 2004; Johnsson et al. 2014). The mortality of Atlantic salmon in the present study was probably mainly caused by predation from grey heron (*Ardea cinerea*), cormorants (*Phalacrocorax carbo*), and adult trout (*Salmo trutta*), which are common in the area. Mortality from starvation is less likely to have occurred because fish were released into the stream shortly before migrating downstream to the sea. Hence, impaired development of anti-predator behaviour in fish reared at high-density offers a potential explanation for the lower survival in the stream. In addition, assuming that predation risk was related to the time that fish spend within the stream, the slightly slower migration time of HD individuals could have increased predator exposure and consequently contributed to the reduced survival.

Another possible explanation for the higher downstream migration success of LD individuals could be that they were at a more advanced stage of smoltification compared to individuals in the HD treatment. This could also explain the small delay in migration time of HD fish. However, because all released fish appeared to be fully smoltified as judged from morphological features (i.e., a silvery appearance, darkened fins, and slim body shape), this scenario is questionable. Previous studies have also been unable to detect effects of rearing densities up to 86 kg fish m^{-3} on gill Na^+,K^+-ATPase activity in Atlantic salmon (Brockmark et al. 2007; Hosfeld et al. 2009), which is widely used as a physiological indicator of smolt status (Hoar 1988; Aarestrup et al. 2000). Nevertheless, Brockmark et al. (2007) found a higher survival of Atlantic salmon that were reared at reduced density after being exposed to a seawater challenge test and visual assessment of body coloration showed that these individuals had a more slivery appearance than those reared at high-density. This indicates that rearing density may influence smolt development (see also Schreck et al. 1985). Thus, further studies involving physiological and morphological indices of smolt status are required to resolve whether the observed difference in migration success among density treatments was mediated by differences in level of smoltification.

In agreement with previous work on salmonids, dorsal fin damage was found to be more severe among fish in the HD treatment (e.g., Latremouille 2003; Brockmark et al. 2007; Kavanagh and Olson 2014). Individuals reared at high-density also exhibited the highest degree of erosion to the opercula. Aggressive acts between individuals have been recognized as the primary cause of dorsal fin damage in hatchery-reared salmonids, making it a useful indicator of aggression levels (Abbott and Dill 1985; Turnbull et al. 1998; Latremoille 2003). Indeed, aggressive bites to the dorsal fin and attacks directed at this area were frequently observed in the present study. The definitive cause of the shortened opercula is less clear

(Kazlauskienė et al. 2006), but agonistic interactions might represent an important factor as well. These findings suggest increased levels of aggression in the HD treatment. Aggressive encounters with conspecifics can be a significant source of stress in hatchery environments (Schreck 1982; Wedemeyer 1997; Näslund et al. 2013), and it is therefore likely that fish in the high-density treatment experienced elevated stress levels relative to those at reduced density. Increased stress levels have previously been shown to decrease survival of smolts during seaward migration and could provide an explanatory factor in the lower survival of HD individuals in the present study (Midwood et al. 2014). Additionally, damaged fins and opercula may decrease swimming performance and increase susceptibility to predation and opportunistic pathogens, with the potential to reduce post-release survival of hatchery fish (Arnold et al. 1991; Ellis et al. 2002; Petersson et al. 2013).

In conclusion, our study shows that the post-release survival of hatchery-reared Atlantic salmon smolts along their migratory pathway to the sea can be significantly improved by reducing rearing density. Density reduction is a simple method to practice in supplementation hatcheries, but some important considerations must be made before implementation. An obvious limitation of the present study is that the adult return rate was not evaluated. The socioeconomic benefits of lowering rearing densities will ultimately depend on whether the increased production costs in the hatchery are compensated by increased number of adult returns. Answering such a question requires long-term studies investigating the smolt-to-adult return ratio and development of economic models. Aside from the economic aspects, there is also a growing public concern, as well as increasingly strict legislation, concerning animal welfare in many countries (Huntingford et al. 2006; Ashley 2007). In this context, the ultimate goal should be to minimize stress and allow natural behaviour to be expressed (Johnsson et al. 2014). Additional studies are also required to determine the generality of the present findings. These studies should ideally be conducted throughout the native distribution of Atlantic salmon and involve a range of different hatchery facilities and natural habitats. Considering that only one of many variables was manipulated in the present study, our results highlight the scope for improving rearing methods of supplementation and/or conservation hatcheries in order to optimize outcomes of releases.

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Table 1. Mean (±SD) total length (mm), body mass (g), and Fulton's condition factor (K) for Atlantic salmon (*Salmo salar*) reared at conventional hatchery density (HD) and one-third of conventional density (LD). Fish were measured at three occasions during the rearing period in the hatchery: 12 July 2012, 5 November 2012, and 20 March 2013. The fish released into Gudsø Stream were measured on 22 March 2013.

	LD				HD			
	n	Length (mm)	Mass (g)	K	n	Length (mm)	Mass (g)	K
Hacthery								
12 July 2012	300	53.7 ± 4.73	1.7 ± 0.49	1.04 ± 0.066	300	52.6 ± 4.65	1.6 ± 0.45	1.04 ± 0.060
5 November 2012	150	85.8 ± 20.16	8.1 ± 5.87	1.07 ± 0.090	150	79.8 ± 15.09	6.0 ± 4.04	1.05 ± 0.078
20 March 2013	150	118.9 ± 26.05	20.7 ± 12.04	1.07 ± 0.077	150	114.9 ± 26.46	18.9 ± 11.99	1.06 ± 0.075
Released fish								
22 March 2013	300	152.2 ± 8.05	31.9 ± 5.44	0.90 ± 0.056	300	147.3 ± 7.12	29.4 ± 4.75	0.91± 0.064

Table 2. Percentage and absolute number (in brackets) of Atlantic salmon (*Salmo salar*) with fin and opercula erosion reared at conventional hatchery density (HD) and one-third of conventional density (LD). The intensity of fin and opercula erosion was quantified using an ordinal scale of 0 (0% eroded), 1 (1-24% eroded), 2 (25-49% eroded), and 3 (>50% eroded)

	Degree of erosion			
	0	1	2	3
Left operculum				
LD	44.7 (67)	54.0 (81)	1.3 (2)	0.0 (0)
HD	27.3 (41)	52.0 (78)	20.7 (31)	0.0 (0)
Right operculum				
LD	84.0 (126)	16.0 (24)	0.0 (0)	0.0 (0)
HD	64.0 (96)	34.0 (51)	2.0 (3)	0.0 (0)
Dorsal fin				
LD	57.3 (86)	31.3 (47)	11.3 (17)	0.0 (0)
HD	50.7 (76)	20.0 (30)	28.7 (43)	0.7 (1)
Left pectoral fin				
LD	100.0 (150)	0.0 (0)	0.0 (0)	0.0 (0)
HD	100.0 (150)	0.0 (0)	0.0 (0)	0.0 (0)
Right pectoral fin				
LD	100.0 (150)	0.0 (0)	0.0 (0)	0.0 (0)
HD	100.0 (150)	0.0 (0)	0.0 (0)	0.0 (0)
Caudal fin				
LD	100.0 (150)	0.0 (0)	0.0 (0)	0.0 (0)
HD	100.0 (150)	0.0 (0)	0.0 (0)	0.0 (0)

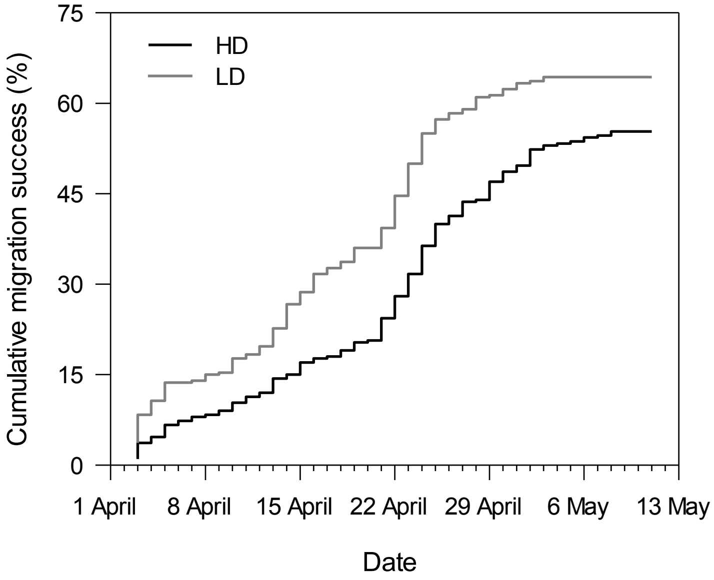

Figure 1. The cumulative migration success (%) in Gudsø Stream for Atlantic salmon (*Salmo salar*) reared at conventional hatchery density (HD) and one-third of conventional density (LD). Three hundred fish from each density treatment were released in the stream on 3 April 2013.

PAPER IV

Effects of emergence time and early social rearing environment on behaviour of Atlantic salmon: consequences for juvenile fitness and smolt migration

Martin H. Larsen, Jörgen I. Johnsson, Svante Winberg, Alexander D.M. Wilson, David Hammenstig, Per-Ove Thörnqvist, Jonathan D. Midwood, Kim Aarestrup, and Erik Höglund

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A newly hatched Atlantic salmon (*Salmo salar*) alevin. Photo: Martin Hage Larsen.

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RESEARCH ARTICLE

Effects of Emergence Time and Early Social Rearing Environment on Behaviour of Atlantic Salmon: Consequences for Juvenile Fitness and Smolt Migration

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Abstract

Consistent individual differences in behaviour have been well documented in a variety of animal taxa, but surprisingly little is known about the fitness and life-history consequences of such individual variation. In wild salmonids, the timing of fry emergence from gravel spawning nests has been suggested to be coupled with individual behavioural traits. Here, we further investigate the link between timing of spawning nest emergence and behaviour of Atlantic salmon (*Salmo salar*), test effects of social rearing environment on behavioural traits in fish with different emergence times, and assess whether behavioural traits measured in the laboratory predict growth, survival, and migration status in the wild. Atlantic salmon fry were sorted with respect to emergence time from artificial spawning nest into three groups: early, intermediate, and late. These emergence groups were hatchery-reared separately or in co-culture for four months to test effects of social rearing environment on behavioural traits. Twenty fish from each of the six treatment groups were then subjected to three individual-based behavioural tests: basal locomotor activity, boldness, and escape response. Following behavioural characterization, the fish were released into a near-natural experimental stream. Results showed differences in escape behaviour between emergence groups in a net restraining test, but the social rearing environment did not affect individual behavioural expression. Emergence time and social environment had no significant effects on survival, growth, and migration status in the stream, although migration propensity was 1.4 to 1.9 times higher for early emerging individuals that were reared separately. In addition, despite individuals showing considerable variation in behaviour across treatment groups, this was not translated into differences in growth, survival, and migration status. Hence, our study adds to the view that fitness (i.e., growth and survival) and life-history

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predictions from laboratory measures of behaviour should be made with caution and ideally tested in nature.

Introduction

In recent years, it has become increasingly recognized that individuals within a population can differ consistently in suites of correlated behavioural traits across time and context. Such individual variation has been reported in a wide variety of animal taxa including fishes [1,2], birds [3,4], mammals [5,6], reptiles [7], amphibians [8] as well as a range of invertebrates [9–11], with the terms temperament, personality, and behavioural syndrome being used to describe this phenomenon [12–15]. In addition, the term stress coping style, characterized along a proactive–reactive continuum, is used for describing individual variation in both behavioural and physiological responses to stress [16]. To date, the majority of studies regarding such individual variation have focused on adult animals, but recently questions have arisen about its existence in early ontogeny and its relation to life-history traits [8,17].

In salmonids, eggs are deposited by the female in gravel nests on the stream bottom. After hatching, the larvae remain within the gravel and feed endogenously on their yolk sac, whereupon they emerge from the spawning nest and start defending feeding territories. The time to emerge from the gravel may vary by several weeks among individuals in the same spawning nest [18,19]. Those that emerge first from the nest are generally considered to have a competitive advantage over later conspecifics because of early access to profitable feeding territories [20–23]. However, this benefit may be offset by costs such as increased predator exposure [24]. These conflicting selective pressures are believed to play an important role in maintaining variability in larval developmental rate in the wild [22].

Several studies have shown that the variation in the timing of spawning nest emergence is related to behavioural traits expressed by the individual [25–28]. For example, Metcalfe and Thorpe [25] demonstrated that early emerging Atlantic salmon (*Salmo salar*) fry were more aggressive and had a higher probability of becoming socially dominant compared to those emerging late. Inherited components underlying this relationship have been demonstrated in two strains of rainbow trout (*Oncorhynchus mykiss*) selected for stress responsiveness (i.e., post stress plasma cortisol; see [29]). The strain exhibiting low stress responsiveness (LR), characterized by having an increased propensity for boldness and social dominance, also showed an earlier emergence time compared to the shy and less socially competitive high stress responsive (HR) strain [28]. These findings indicate a relationship between stress coping styles and timing of spawning nest emergence in salmonid fishes [25,28].

Although consistent individual variation in behaviour is now well documented in several fish species (reviewed in Conrad et al. [1]), remarkably little is known about whether and to what extent behavioural traits expressed early in ontogeny influence fitness (e.g., growth and survival) and life-history trajectories. In addition, studies of behavioural trait variation have mainly been conducted under laboratory conditions and the ecological consequences of consistent individual differences in behaviour for fish in the wild remains unclear [30,31]. Salmonids represent excellent candidates for addressing such questions as they usually exhibit extensive variation in behavioural and life-history patterns such as age at sexual maturity and sea migration [32]. While some studies of juvenile salmonids have demonstrated that fitness and life-history strategies may be linked to individual differences in behavioural traits [25,33–35], others have been unable to document such relationships [36,37]. Moreover, it has been suggested that behavioural characteristics associated with spawning nest emergence can affect growth and timing of smoltification (seawater adaptation) in salmonids reared under hatchery conditions [25,38]. However, it remains unknown if such relationships persist in natural habitats, where environmental complexity is higher and there are more dynamic ecological factors influencing selection such as competition, predation, and resource availability.

A study on the HR and LR rainbow trout strains also suggest effects of the social environment on fitness-related traits [39]. When co-reared, the more bold and aggressive LR strain outgrew the HR strain, an effect not seen when these two strains were reared separately. Additionally, several studies suggest that behavioural traits may be plastic and sensitive to social conditions [40,41]. Studies on rainbow trout, for example, show that previous conspecific experience may alter the degree of boldness and aggression of individuals [41,42]. Magnhagen and Staffan [40] demonstrated that individual young-of-the-year perch (*Perca fluviatilis*) can adjust boldness and exploration tendency relative to their social group. However, there is a shortage of studies that elucidate the effects of social environment early in ontogeny in shaping an individual's behavioural trait composition and its potential for affecting fitness and life-history trajectories.

Knowledge of how individuals with different behavioural traits perform in nature is crucial for incorporating the framework of consistent behavioural variation into management practices [1,30]. This requires that high throughput methods for characterizing behavioural trait composition must be developed. As mentioned previously, there seems to be a relationship between emergence time from spawning nests and the behavioural profile of an individual. Therefore, sorting salmonid fishes according to larval developmental rate in the hatchery may offer such a method for behavioural characterization. Potentially, this will generate knowledge about the linkage between larval developmental rate and behavioural trait compositions, which may be applied in restocking strategies. In this respect, it is equally important to understand the potential impacts of social environments on behavioural expression during the early rearing phase before releasing the hatchery fish into the wild.

In the present study, our primary objectives were to investigate if the relationship between behavioural traits and the time of emergence is affected by the social rearing environment, and to investigate whether trait compositions expressed in the hatchery are related to growth, survival, and migration status in natural environments. To assess this, Atlantic salmon fry were sorted with respect to time of emergence into three groups: early (first one-third), intermediate (middle one-third), and late (last one-third). To investigate social effects on behavioural traits, these groups were reared separately or in co-culture for four months. After this, the behaviour profile of individuals with different emergence time and rearing environment where characterized by a basal locomotor activity test [34], a novel object test [43], and a net restraining test [44]. Following behavioural characterization, fish were released into a closed near-natural experimental stream to examine the potential effects of differences in emergence time and previous rearing environment on fitness (i.e., growth and survival) and smolt migration.

Materials and Methods

Ethics statement

This study was carried out in strict accordance with the recommendations of the Swedish Board of Agriculture and conformed to the national guidelines of Sweden. All animal procedures were approved by the Ethical Committee for Animal Research in Gothenburg (Dnr 8-211). All efforts were made to minimize animal handling and stress.

Experimental fish and housing conditions

The fish used in the present study were first-generation offspring (F1) of Atlantic salmon originating from the River Dalälven in central Sweden. Since the construction of a power plant in 1915 in Älvkarleby, located about 10 km from the river mouth to the Gulf of Bothnia, anadromous fish have been prevented from completing their upstream migration to spawning grounds beyond the dam. To compensate for the loss of natural production of Atlantic salmon, mature adults are caught annually in the river and artificially crossed in order to create supportive stocks. The offspring are hatchery-reared and released as 1- and 2-year-old smolts in the river during spring. Although Atlantic salmon spawn naturally in the river below the dam, wild smolt production in this system is minimal, representing less than 1% of total hatchery supplementation [45]. In this study, the parental fish were caught in a permanent fish-trap situated at the upper end of Kungsådran (a 1.6 km long branch of River Dalälven) during the autumn of 2011. The fish were transported to the Fishery Research Station in Älvkarleby and kept in indoor concrete ponds. On the 7th of November 2011, eggs from 24 females (73–104 cm in total length) were stripped and fertilized with the milt from 24 males (71–107 cm in total length). Eggs from each female were fertilized by a haphazardly chosen male and incubated separately in hatching trays. At 141 day degrees post fertilization (DDF), eyed eggs were placed into eight artificial spawning nests, which allowed fry to be sorted with respect to emergence time. Each spawning nest contained 600 eggs originating from three different females (i.e., 200 eggs from each female). The spawning nests consisted of three parts: a flat holding tray with golf balls as bottom substrate to mimic natural gravel, an emergence route, and a collection tank (see [27]). The nests were continuously provided with water from River Dalälven at ambient temperature (mean: 7.1°C; range: 2.4–15.7°C) and the photoperiod followed natural daylight cycles. After hatching, the larvae laid dormant and undisturbed until they reached an appropriate developmental stage and started to emerge from the artificial gravel in search of food. During the period of emergence, the fry were transported downstream by a water current to the emergence route which ended up in the collection tank. Fry were removed daily from the collection tanks and divided into three different emergence groups: early (first 200 fish), intermediate (middle 200 fish), and late (last 200 fish). The emergence period was initiated on the 4th of May 2012 (at 338 DDF) and after 24 days all individuals had left the spawning nests (at 611 DDF). The overall mortality rate was low (4%) during this period. Fry from the eight spawning nests were held in one of three tanks based on their emergence time (i.e., early, intermediate, and late). After all fry had emerged, individuals from each emergence group were collected and haphazardly divided among four replicate tanks (300 L, 110 × 110 × 25 cm) such that there were 370 fish in each tank. The fry were fed commercial trout pellets (Aller Performa, Aller Aqua, Denmark) with an automatic feeder.

Between the 12th and14th of June 2012, the fish were marked with different colored visible implant elastomers (VIE) according to emergence fraction (fluorescent red, green, and blue; Northwest Marine Technology, Washington, USA). Fish were anaesthetized (benzocaine 20 mg L^{-1}) and placed on a wet tissue under a stereomicroscope. The marker was injected in the adipose eyelid tissue behind the eye at a shallow angle using a 29 gauge needle. When applied properly, VIE-tagging has no measurable effects on growth and survival of small salmonids [46]. A haphazardly collected subsample of fish from each of the three emergence groups was preserved in ethanol (80%) to determine length and body mass at time of VIE-tagging ($n = 30$ for each emergence group). Overall, the average fork length (FL) and mass of these preserved individuals was 30.6 ± 0.171 (SE) mm and 0.19 ± 0.004 (SE) g, respectively, and no differences were found among the emergence groups (generalized linear model; length: $F = 0.926$, $df = 2$, $p = 0.400$; mass: $F = 1.077$, $df = 2$, $p = 0.345$).

To investigate social rearing effects of differences in emergence time, fish with different emergence times were reared either separately (mono-culture) or mixed (co-culture) for approximately four months post-emergence. This resulted in four different rearing groups (early, intermediate, late, or mixed emergence time) that were each replicated three times. The mixed group replicates each consisted of 300 individuals (100 individuals from each of the three emergence groups) and the replicates for the separately reared groups each consisted of 300 individuals per emergence group. Fish from each emergence group were randomly selected from the four replicate tanks in equal numbers. Each rearing group was reared in three replicate tanks (300 L, 110 × 110 × 25 cm; 12 tanks in total) supplied with river water (Dalälven) and kept under natural photoperiod and temperature (mean: 15.1°C; range: 5.9–20.0°C) conditions. Fish were fed daily during daylight hours with commercial trout pellets at a ratio equivalent to 2% of body mass using automatic feeders. The pellet size was changed in accordance to the manufacturer's recommendations as the fish increased in size.

Behavioural tests

Between the 11th and 26th of October 2012, the individual behaviour of 20 fish from each of the six treatment groups consisting of the three emergence groups (early, intermediate, and late) reared under two conditions (mono-culture and co-culture) was scored (i.e., 120 fish in total). The 20 individuals in each treatment group were haphazardly collected from each of the three replicate tanks in roughly equal numbers (i.e., between 6–7 fish from each tank). However, to avoid potential adverse effects of PIT-tagging (see below) on growth and survival only fish above 85 mm FL were included in the experiment [47]. Behavioural trials (n = 15) occurred in batches of eight fish. Each fish was transferred to one of eight experimental tanks (opaque white plastic tanks, 55 × 36 cm, water level 6.5 cm) in the afternoon (at 17:00) prior to the day of behavioural observations. Hence, fish could not see one another during the behavioural tests. The tanks were arranged in two rows of four units which were positioned underneath two video cameras to allow simultaneous recording. To avoid potential tank effects, we randomized allocation of the fish from the different treatment groups between the experimental tanks. In addition, at least one fish from each treatment group was represented during each trial to minimize time effects. The next day, the behaviour of each fish was scored in each of the following contexts: (1) a basal locomotor activity test (adapted from [34,48]), (2) a novel object test (adapted from [34,41,43]), and (3) a net restraining test (adapted from [44,49,50]; Fig. 1). These assays were chosen because they characterize important aspects of the behaviour of juvenile Atlantic salmon, such as basal activity pattern, boldness, and their escape response. All behavioural tests were carried out between 09:00 am and 12:15 pm on the day following transfer to the experimental set-up and the timing of all observations was similar among trials. The entire experimental arena was surrounded by an observation blind constructed from green tarpaulin, ensuring that fish were not disturbed during behavioural observations. The experimental tanks were supplied with flow-through river water at a flow rate of 2.6 L min^{-1}, corresponding to approximately 12 cycles per hour. The water temperature ranged between 5–8°C and the tanks were exposed to a 9 h light:15 h dark cycle with day break set at 07:00 am. Illumination was provided by two lamps (Walimex Daylight 1000, Burgheim, Germany) and the light intensity was 160 lux. Experimental tanks were cleaned with ethanol (95%) and subsequently flushed with river water between each trial. Fish were not fed after being moved to the behavioural observation tanks.

Basal locomotor activity test. In the first test, the undisturbed swimming pattern (locomotor activity) of the juvenile Atlantic salmon was video recorded for 30 min in the experimental tanks (Fig. 1). Based on interpretation of the videos, the following information for each

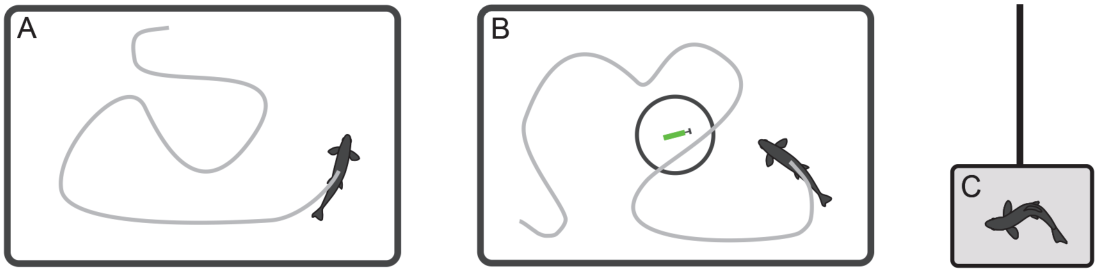

Fig 1. Schematic representation of the experimental set-up for the measurements of individual behavioural traits. (A) basal locomotor activity test, (B) novel object test, and (C) net restraining test. The novel object zone is indicated by the circle. The different elements are not to scale.

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individual was recorded: average swimming velocity (cm/s), total time (s) with velocity greater than 1 cm/s (i.e., total time active), and maximum recorded velocity (cm/s).

Novel object test. Following the basal locomotor activity test, the tendency of the fish to explore a novel object was measured (Fig. 1). The novel object test has been widely used in behavioural research to assess boldness in fish (e.g., [41,43]). The object consisted of a bright-green rawlplug (3.5 cm long and 0.8 cm in diameter) with a stainless screw (served as ballast). The object were attached to a transparent fishing line and gently lowered down in the center of the experimental tank. The 30 min observation period started after the novel object was lowered to the bottom of the tank. A radius of 5 cm around the novel object was defined as the novel object zone. The following behaviours were recorded for subsequent analyses: latency to enter the zone (s), total number of entries into the zone, total time spent within the zone (s), and average distance to the zone (cm). Entrance into the novel object zone was defined when the center-point of the fish was inside the area. The latency was set to 1800 s if the fish did not enter the zone. Any fish that was positioned in the novel object zone prior to the object being lowered was excluded from further analysis of boldness (n = 1).

Net restraining test. The net restraining test has been shown to correlate with physiological and behavioural traits in different fish species, and it has been previously used to distinguish individuals with contrasting stress coping styles; the proactive and reactive stress coping styles [44,49,50]. Each fish was transferred from the experimental tanks following the novel object test to an aerated bucket (15 L) covered with a lid. After a 30 min resting period, the fish was gently poured from the bucket into a net, mounted on a retort stand at an angle of 45°. While in the net, the total time spent on escape attempts (s) was recorded for 60 s. An escape attempt was defined as a tail beat. If for some reason a test fish could not be transferred within a standardized 10 s protocol for the restraining test, it was excluded from further analyses of escape behaviour (n = 3).

After completion of the net restraining test, fish were anaesthetized and surgically implanted with 12.5 mm PIT tags (ISO FDX-B, 134.2 kHz, diameter 2.1 mm; Biomark, Boise, Idaho, USA) to allow for individual recognition. The PIT tag was inserted into the peritoneal cavity through an approximately 2 mm long ventrolateral incision made slightly anterior to the muscle bed of the left pelvic fin. The tagging incision was left to heal without closure [51]. Fork length and mass of the fish were measured to the nearest 1 mm and 0.1 g, respectively. There were no significant differences in length and mass among fish scored for behaviour in the six treatment groups (generalized linear model; length: F = 0.178, df = 5, p = 0.970; mass: F = 0.378, df = 5, p = 0.863; Table 1).

Following PIT-tagging, the fish scored in the behavioural trials were returned to new holding tanks until they were released in an experimental stream (see below). Individuals with early, intermediate, and late emergence time reared in mono-culture were kept in three

Table 1. Fork length and mass of the Atlantic salmon (*Salmo salar*) among experimental treatments (n = 20 for each treatment) measured directly after the behavioural assays.

Treatment	Length (mm)		Mass (g)	
	Mean ± SE	Range	Mean ± SE	Range
Mono-culture				
Early	99.5 ± 1.06	91–108	10.5 ± 0.31	7.9–13.6
Intermediate	99.8 ± 1.18	91–110	10.7 ± 0.33	7.8–14.0
Late	98.3 ± 0.97	89–105	10.1 ± 0.27	7.5–11.7
Co-culture				
Early	99.3 ± 1.15	88–110	10.2 ± 0.42	6.9–15.0
Intermediate	99.3 ± 1.29	90–108	10.4 ± 0.40	7.4–13.5
Late	99.2 ± 1.19	89–112	10.3 ± 0.38	7.6–14.8

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separate tanks (200 L, 100 × 100 × 20 cm) and the mixed emergence time group was reared in a larger tank (600 L, 100 × 200 × 30 cm). Hence, the density of fish from the different treatments groups was similar in terms of number fish per cubic meter. All fish were in good condition after tagging and no tags were rejected in the tanks.

Performance in the stream

On the 7th of November 2012, the 120 fish scored in the behavioural trials were introduced into an experimental stream in Älvkarleby to compare growth, survival, and migration status (resident or migrant) of the different treatment groups under near-natural conditions (see [52] for a schematic view of the experimental stream). A tube supplies the stream with river water from the nearby hydro-electric dam in River Dalälven. The experimental stream has a length of 110 m and a total area of 345 m^2. Wolf traps are situated at the upper and lower end of the stream and all water is running through the traps. A system of stainless gratings leads the fish to a collection tank (mesh size: 1 × 1 mm). Hence, no Atlantic salmon can escape from the stream, nor can any fish invade the system. The experimental stream consists of four pools with three riffles in between. The maximum water depth of the pools ranges from 0.8 to 1.2 m. The bottom substratum varies from mud, through coarse sand, to gravel, and larger stones. During the experiment the adjustable water flow was set to a maximum of 150 L s^{-1} and the average water temperature in the stream was 2.7°C (range: 0.1–18.0°C).

Fish were haphazardly released in equal numbers into the two middle pools of the stream. The density of Atlantic salmon in the stream corresponded to 35 individuals per 100 m^2. This density is within the range of natural population densities of juvenile Atlantic salmon in the region [53]. Throughout the entire experiment, the downstream and upstream trap was inspected daily for the presence of Atlantic salmon. No fish were caught in the upstream trap. Length and mass of descending fish were measured and their PIT tag number was recorded using a handheld reader (Biomark 601, Boise, Idaho, USA). On the 4th of June 2013, approximately seven months after fish were released, the experimental stream was drained slowly to recover surviving fish that remained in the system. All fish were measured, weighed, and identified. Fish only had access to naturally occurring food items, and were exposed to natural predation from American mink (*Mustela vison*) and gray heron (*Ardea cinerea*). For information on important prey taxa in the stream, see Johnsson et al. [52].

Data handling and statistics

Video analyses. Recorded videos of basal locomotor activity and novel object test were analyzed with the video-tracking software EthoVision XT 10 (Noldus Information Technology, Wageningen, Netherlands) at a recording rate of 5 frames per second. This software distinguishes fish from their ambient background on the basis of their greyscale/brightness values. Center-point detection of the fish was chosen as the default setting in all tests and tracks were smoothed using the LOWESS algorithm. After the analysis was complete, the tracks were visually inspected for artifacts. Subject loss due to misdetections by video-tracking software was $<0.7\%$. The recorded escape response behaviour of the Atlantic salmon in the net restraining test was analyzed manually using a media player (SMPlayer for windows version 0.8.6). The video recordings were played at 0.5 × normal speed during analyses.

Behaviour. Separate principal component analyses (PCAs) were used to summarize the behavioural measures for the basal locomotor activity test (i.e., average swimming velocity, total time active, and maximum recorded velocity) and the novel object test (i.e., latency to enter the zone, total number of entries into the zone, total time spent within the zone, and average distance to the zone). Each PCA resulted in one main principal component (PC) with eigenvalue greater than one (Kaiser-Guttman criterion), further referred to as PC activity and PC boldness respectively. The correlation matrix was used to check multicollinearity of variables. Kaiser-Meyer-Olkin (KMO) test for sample adequacy was greater than 0.6 and the Bartlett's test of sphericity was significant for all tests ($p < 0.0001$) [54]. Only variables with loadings $>|0.4|$ were considered to be important in constructing a principal component (sensu [54]). The extracted component scores (i.e., PC activity and PC boldness) were used as representative behavioural scores in subsequent analyses. To analyze effects of emergence time, rearing environment, and fish length on PC activity and PC boldness, two separate GLMs (generalized linear model) with Gaussian distribution and identity link function were used according to the following model (M1):

$$\begin{aligned}&\text{Response variable}\\&\quad = \text{intercept} + \text{emergence time} + \text{rearing environment} + \text{length} + (\text{emergence time}\\&\qquad \times \text{ rearing environment})\end{aligned}$$

with emergence time (early, intermediate, and late) and rearing environment (mono-culture and co-culture) as fixed factors. Fork length measured directly after the behavioural tests was included as covariate to correct for potential effects of body size. M1 was also used to analyze the duration of escape attempts in the net restraining test. Across experimental treatment groups, correlations between PC activity, PC boldness, and duration of escape attempts during net restraining were assessed using non-parametric Spearman rank correlations because these variables were not all normally distributed.

Performance in the stream. Survival rate in the experimental stream was calculated as the number of fish recovered in the stream at the end of the experiment plus the fish caught in the downstream trap divided by the total number of fish released in the stream multiplied by 100. The percentage of fish that migrated was calculated as number of fish caught in the downstream trap divided by the total number of fish released in the stream multiplied by 100. GLMs with binomial distribution and logit link function, also known as logistic regression, were used to model survival and downstream migration probabilities in the experimental stream, including the same variables as in M1. Likelihood ratio tests (LRT) were used to evaluate overall model significance. Specific growth rate (SGR; g day^{-1}) for mass was calculated according to the following formula:

$$SGR = (\log_e M_f - \log_e M_i) \times t^{-1}$$

where M_i and M_f are the initial and final mass (g), respectively, and t is the time in days. A GLM with Gaussian distribution and identity link function was used to model the average SGR for Atlantic salmon among the treatment groups according to M1. Effects of PC activity, PC boldness, and duration of escape attempts during net restraining on the probability of survival and migration in the stream were investigated using separate GLMs with binomial distribution and logit link function. Finally, the correlation of SGR with PC activity, PC boldness, and duration of escape attempts was assessed using Pearson's correlation.

Statistical software, data exploration, and model assumptions. Initial data exploration was based on the protocol in Zuur et al. [55]. Cleveland dotplots, boxplots, and multipanel scatter plots were used to identify outliers and to examine relationship between variables. Assumption of homogeneity of variance and normal distribution for the GLM models was ensured by visual inspection of residual plots, and Cook's distance was calculated to detect any values exerting extreme influence on the model fit. PC activity and PC boldness required a rank transformation to satisfy the assumption of normality. The interaction between emergence time and rearing environment was excluded from the final model if $p > 0.05$. In case of a significant main term, Tukey honestly significant difference (HSD) test was employed to investigate differences among treatment groups. All statistical analyses were performed in R 3.0.1 (R Development Core Team 2013) and SPSS 20.0 (IBM Corporation, Armonk, NY, USA). Variation in association with recorded mean values is given as standard error throughout. Statistical significance for all analyses was assessed at an alpha level of 0.05.

Results

Behaviour

Separate PCAs on the behavioural measures for the basal locomotor activity and novel object test each extracted one main principal component, further referred to as PC activity and PC boldness respectively (Table 2 and 3). Average swimming velocity, total time active, and maximum velocity contributed to PC activity, with high positive loadings for all variables (Table 3); individuals that scored highly on this component had high levels of activity. PC boldness reflects the degree of boldness displayed by an individual and it was dominated by three variables; number of entries and duration of visits to the novel object zone were positively associated with one another, but negatively associated with the latency to enter the zone (Table 3). Therefore, high values on this component indicate bolder individuals. Average scores for PC activity and PC boldness were not affected by emergence time, rearing environment, or their interaction (GLM; $F \leq 2.110$, df = 1–2, $p \geq 0.126$). However, smaller fish generally had higher scores for PC boldness than larger conspecifics (GLM; F = 5.164, df = 1, p = 0.025).

Loadings, eigenvalues, and explained variance (%) were calculated separately for behaviours describing basal activity (PC activity, n = 120) and boldness (PC boldness, n = 119). Loadings with absolute value above 0.4 are shown in bold.

Regardless of previous rearing environment, the duration of the escape attempts in the net differed significantly among the three emergence groups (GLM; F = 3.133, df = 2, p = 0.047; Table 2; Fig. 2). Post hoc comparisons demonstrated that early emerging Atlantic salmon spend less time escaping compared to those with an intermediate emerging time (Tukey HSD test; p = 0.044), but no difference was detected between early and late emerging fry (Tukey

Table 2. Descriptive values (mean ± SE) for scores of behavioural traits in each of three behavioural contexts: (1) basal locomotor activity test, (2) novel object test, and (3) net restraining test.

Context/score	Mono-culture			Co-culture		
	Early	Intermediate	Late	Early	Intermediate	Late
Basal locomotor activity test						
Average velocity (cm/s)	2.4 ± 0.21 (20)	1.5 ± 0.29 (20)	1.9 ± 0.26 (20)	2.0 ± 0.43 (20)	2.0 ± 0.32 (20)	2.1 ± 0.24 (20)
Total time active (s)	651.9 ± 52.77 (20)	404.0 ± 73.86 (20)	514.8 ± 66.76 (20)	488.9 ± 87.15 (20)	537.4 ± 80.13 (20)	563.9 ± 62.82 (20)
Maximum velocity (cm/s)	12.8 ± 0.61 (20)	12.7 ± 1.36 (20)	12.1 ± 1.24 (20)	12.2 ± 0.92 (20)	12.2 ± 0.82 (20)	12.5 ± 0.74 (20)
Novel object test						
Total time in the zone (s)	3.8 ± 1.39 (20)	5.0 ± 2.30 (20)	4.5 ± 1.73 (20)	10.6 ± 6.52 (19)	3.4 ± 1.46 (20)	4.6 ± 1.46 (20)
Number of zone entries	1.8 ± 0.52 (20)	1.6 ± 0.51 (20)	1.7 ± 0.60 (20)	2.1 ± 0.73 (19)	1.8 ± 0.88 (20)	1.8 ± 0.54 (20)
Latency to enter the zone (s)	1209.0 ± 154.71 (20)	1385.1 ± 119.54 (20)	1400.6 ± 136.38 (20)	1198.1 ± 140.80 (19)	1379.1 ± 141.38 (20)	1185.6 ± 157.09 (20)
Average distance to the zone (cm)	15.3 ± 1.03 (20)	17.0 ± 0.84 (20)	16.0 ± 1.00 (20)	14.2 ± 1.14 (19)	17.1 ± 0.69 (20)	14.7 ± 0.99 (20)
Net restraining test						
Total escape time (s)	17.5 ± 1.99 (20)	22.7 ± 2.21 (18)	21.9 ± 2.30 (19)	18.8 ± 2.21 (20)	23.7 ± 2.32 (20)	21.8 ± 1.79 (20)

Sample sizes are given in parentheses.

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HSD test; $p = 0.204$). The duration of escape attempts was also similar between intermediate and late emerging individuals (Tukey HSD test; $p = 0.752$).

PC activity and PC boldness was positively correlated with one another, indicating an activity–boldness syndrome (Spearman correlation; $r_s = 0.375$, $p < 0.0001$, $n = 119$). The duration of escape attempts during net restraining was not associated with either PC activity (Spearman correlation; $r_s = 0.014$, $p = 0.882$, $n = 117$) or PC boldness (Spearman correlation; $r_s = -0.114$, $p = 0.123$, $n = 116$).

Table 3. Results of principal component analyses (PCAs) on behavioural traits in each of two behavioural contexts: (1) basal locomotor activity test, and (2) novel object test.

Principal components	PC
PC activity	
Behaviour	
Average velocity (cm/s)	**0.96**
Total time active (s)	**0.97**
Maximum velocity (cm/s)	**0.81**
Eigenvalue	2.51
% variance explained	83.58
PC boldness	
Behaviour	
Total time in the zone (s)	**0.80**
Number of zone entries	**0.87**
Latency to enter the zone (s)	**–0.89**
Average distance to the zone (cm)	–0.23
Eigenvalue	2.22
% variance explained	55.58

doi:10.1371/journal.pone.0119127.t003

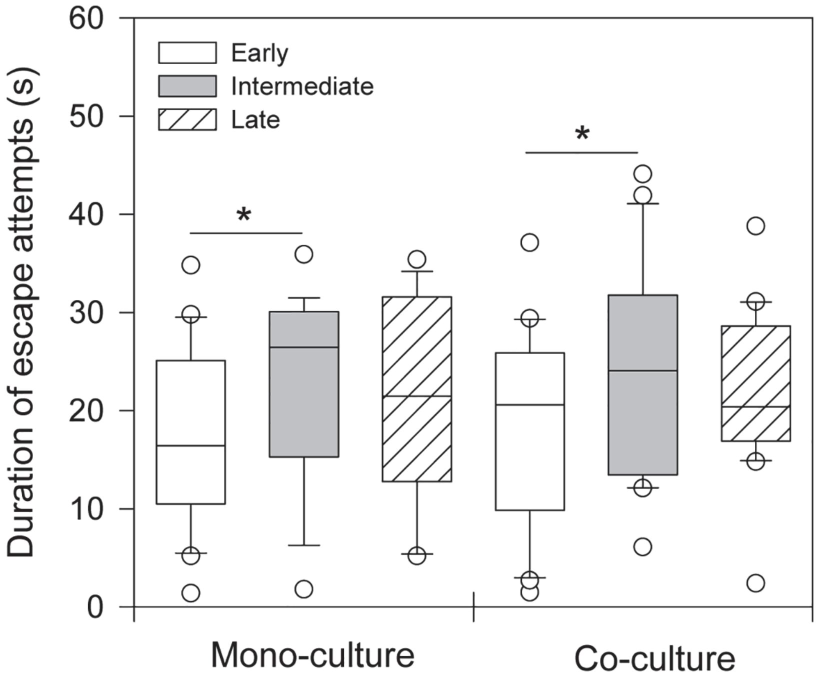

Fig 2. Duration of escape attempts (s) of Atlantic salmon (*Salmo salar*) during a net restraining test. Fish were sorted according to emergence time from artificial spawning nests into three groups: early (first one-third), intermediate (middle one-third), and late (last one-third). These emergence groups were hatchery-reared separately (mono-culture) or together in a mixed population (co-culture) for approximately four months prior to the behavioural assays. Horizontal lines within each box represent median duration of escape attempts (s), ends of boxes represent the 25th and 75th percentiles, and whiskers represent the 10th and 90th percentiles. Open circles indicate outliers outside the 10th and 90th percentiles. The asterisks denote a significant difference at $p < 0.05$ (Tukey honestly significant difference). See Table 2 for sample sizes.

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Performance in the stream

Survival. The survival rate of the Atlantic salmon in the experimental stream ranged from 50% to 70% among experimental treatment groups (Table 4). Mortality in the stream was likely caused by predation from mink and heron, which are common in the area. Starvation-related mortality is also thought to have occurred since ten fish were found dead in the stream from

Table 4. Survival, migration, and specific growth rate (SGR) for Atlantic salmon (*Salmo salar*) among experimental treatment groups in the experimental stream.

Treatment	Survival (%)	Migration (%)	SGR (g day^{-1})
Mono-culture			
Early	70 (14)	65 (13)	0.0025 ± 0.0003 (14)
Intermediate	60 (12)	35 (7)	0.0034 ± 0.0006 (12)
Late	70 (14)	40 (8)	0.0036 ± 0.0006 (14)
Co-culture			
Early	60 (12)	40 (8)	0.0032 ± 0.0006 (12)
Intermediate	50 (10)	45 (9)	0.0027 ± 0.0005 (10)
Late	50 (10)	40 (8)	0.0032 ± 0.0003 (10)

Values are mean ± SE for SGR. Sample sizes are given in parentheses.

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the 1st of March to the 28th of April 2013. These ten fish were in generally poor condition and did not have any obvious injuries, further supporting this assertion of starvation mortality. A binomial GLM revealed no significant effect of emergence time (GLM; LRT = 0.861, df = 2, p = 0.650), rearing environment (GLM; LRT = 2.258, df = 1, p = 0.133), length (GLM; LRT = 0.203, df = 1, p = 0.652), or the combined effect of emergence time and rearing environment (GLM; LRT = 0.309, df = 2, p = 0.857) on the probability of survival.

Growth. The mean specific growth rate (SGR; g day^{-1}) of the Atlantic salmon was not related to emergence time, rearing environment, or their interaction (GLM; $F \leq 0.702$, df = 1–2, $p \geq 0.500$; Table 4), however, smaller fish generally had higher SGR than larger individuals (GLM; F = 10.352, df = 1, p = 0.002).

Migration status. In total, 53 of 120 fish were captured in the downstream trap, giving an overall migration rate of 44% (Table 4). These downstream migrations occurred from the 8th of May to the 3rd of June 2013. There was a peak in the downstream descent that occurred from the 17th of May to the 30th of May, wherein 75% of the migrating fish were found in the trap. Although the rate of migration was particularly high for early emerging fry that were reared separately (65%), the analysis revealed no significant effect of emergence time (GLM; LRT = 1.638, df = 2, p = 0.441), rearing environment (GLM; LRT = 0.331, df = 1, p = 0.565), or their interaction (GLM; LRT = 2.759, df = 2, p = 0.252) on the probability of migration. Migration propensity was positively related to length (GLM; LRT = 5.864, df = 1, p = 0.016).

Behaviour and performance in the stream

The probability of survival and migration in the stream was not related to PC activity, PC boldness, or duration of escape attempts during net restraining (GLM; $LRT \leq 0.575$, df = 1, $p \geq 0.448$). Furthermore, SGR was not associated with these behavioural measures (Pearson's correlation; $p \geq 0.422$).

Discussion

Behaviour

In the present study, the basal locomotor activity was positively correlated with boldness across experimental treatment groups, supporting observations that behavioural syndromes develop early in life of salmonid fishes [33,34]. The noted relationship between boldness and activity is similar to what has been reported in other fishes [48,56]. There was also a relationship between timing of emergence from the artificial spawning nests and behavioural response to the net restraining test. Fry with an early emergence time spent less time on escape attempts in the net compared to intermediate individuals (Fig. 2). The net restraining test has previously been used to discriminate individuals with contrasting stress coping styles [44,49,50]. For example, Gilthead seabream (*Sparus aurata*) that exhibit high activity during net restraining trials share traits characteristics of the proactive stress coping style, including greater risk-taking behaviour and fight-flight response to challenges [44]. If this relationship is present in Atlantic salmon, our results suggest that individuals with an intermediate emergence time have greater resemblance to the proactive coping style than early emerging conspecifics. This interpretation, however, is in contrast to earlier studies of wild populations of Atlantic salmon and selected rainbow trout strains, which have indicated a link between early emergence and the proactive stress coping style [25,26,28]. Moreover, basal activity and boldness were not related to emergence time, raising questions about the link between larval development rate and stress coping styles in this population of Atlantic salmon. Hence, an alternative explanation for the longer escape duration in fish with an intermediate emergence time might be that these individuals have higher physical stamina. The fact that the net escape response behaviour was unrelated to the activity–boldness syndrome also suggests that escape behaviour in Atlantic salmon represent a separate behavioural dimension. This adds to the view that suites of behavioural traits may be organized in independent dimensions [14,57]. Alternatively, this result might have to do with the chosen metric and other escape response metrics (e.g., predator escape) might correlate with the activity–boldness syndrome. Further studies are required to verify whether these behavioural axes remain stable over time and across different situations in nature, as previously demonstrated in other fish species [1].

Social interactions with conspecifics can affect behavioural traits of an individual [40–42]. For instance, bold rainbow trout may reduce their boldness after observing shy demonstrators [41], and European perch can adjust their level of boldness and exploration tendency to that of their social group [40]. In the present study, however, we could not detect any effect of co- or mono-rearing on the behaviour displayed by individuals with different emergence time. Earlier studies have suggested that social effects on behavioural development may be density-dependent, and it is possible that the relatively high rearing densities in this study (~ 250 fish m^{-2}) might not have facilitated social effects on behavioural expression [58,59]. Social behaviour is facilitated by the development of familiarity with other individuals over time, which in turn is limited by the number of individual identities that can be learned or memorized [60,61]. Hence, in a high-density environment there is little scope to develop social relations with specific individuals, which may have impaired the effects of social environment on behavioural development in our study [58].

Performance in the stream

The results of the present study showed no significant effects of emergence time from artificial spawning nests and social rearing environment on growth, survival, and migration status of Atlantic salmon in the experimental stream. However, it is possible that due to the relatively low statistical power in this study we might have been unable to detect some correlations between performance in the stream and experimental treatment groups. For instance, albeit non-significant, it is worth noting that the downstream migration rate was 1.4 to 1.9 times higher for fish with an early emergence that were reared in mono-culture compared to the other treatments (Table 4). Similar results was reported by Metcalfe and Thorpe [25], demonstrating that a higher proportion of early emerging Atlantic salmon fry reached body size thresholds for smoltification during their first summer in the hatchery compared to those emerging later. This was ascribed to be a result of increased propensity for being socially dominant and higher metabolic rates among early emerging fry [25,26]. It should be noted that fish with different emergence times were reared in the same hatchery tank in the study by Metcalfe and Thorpe [25]. Taken together, these results indicate a link between migration status, emergence time, and social rearing environment, and we advocate more studies addressing this issue.

Although the size range of fish in this study was relatively small (i.e., 88 to 112 mm FL), body length at release into the stream was negatively correlated with SGR and positively correlated with migration propensity in the stream, consistent with previous studies on salmonids [62]. Our results also show that length was negatively related with boldness, suggesting that smaller fish were bolder than larger ones. Because smaller salmonids generally have higher mass specific metabolic rate and smaller energy reserves [63], and hungrier individuals are usually more willing to take risks [64,65], increased feeding motivation may have made smaller individuals appear bolder in the novel object test.

Previous studies on fitness (i.e., growth and survival) and life-history consequences of behavioural traits in salmonids have generated inconsistent results [1,30]. While some studies have demonstrated a link between behaviour, growth, and survival [33–35], others challenge the view of such relationships [36,37], emphasizing that the consequences of a given behavioural type on individual growth and survival are likely to differ across environmental context [1]. Although individual fish showed considerable variation in basal activity, boldness, and their escape response during net restraining in the current investigation, none of these behaviours were associated with growth, survival, or migration status under near-natural conditions. Since neither the persistence of behavioural expression over time and across context was examined here, context specificity or instability of behavioural traits cannot be ruled out as a possible explanation for the absence of a relationship between performance in the experimental stream and individual behavioural variation. For instance, unstable behavioural types could emerge if individual behavioural profiles measured in the hatchery were different from those expressed in the stream. It should be noted that we were also restricted in our sample population to fish larger than 85 mm FL among our emergence groups due to potential adverse effects of PIT-tagging on fish growth and survival [47]. Because of this limitation, it is possible that we omitted or reduced representation of certain behavioural phenotypes in our study, which might have contributed to the missing link between behaviour and performance in the stream. Hence, the present results add to the view that the link between behavioural expression from laboratory measures and subsequent performance in nature is often flexible and probably dependent on specific environmental conditions [1,30,66]. Additional empirical studies are needed to clarify the relationship between behavioural traits, fitness (i.e., growth and survival), and life-history. These studies should preferably be performed in a range of different natural habitats to elucidate whether and why some behavioural types are better suited to a given environment than others. From a more applied perspective, our results highlight the challenges associated with incorporating individual variation in behavioural traits into fisheries management and conservation.

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Author Contributions

Conceived and designed the experiments: JIJ SW. Performed the experiments: MHL DH P-OT. Analyzed the data: MHL JIJ. Contributed reagents/materials/analysis tools: JIJ. Wrote the paper: MHL JIJ SW ADMW JDM KA EH.

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PAPER V

Effects of passive integrated transponder tags on survival and growth of juvenile Atlantic salmon *Salmo salar*

Martin H. Larsen, Aske N. Thorn, Christian Skov, and Kim Aarestrup

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23 mm passive integrated transponder (PIT) tags. Photo: Martin Hage Larsen.

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Effects of passive integrated transponder tags on survival and growth of juvenile Atlantic salmon *Salmo salar*

Martin H Larsen*, Aske N Thorn, Christian Skov and Kim Aarestrup

Abstract

Background: A laboratory experiment was conducted to assess the potential impacts of surgically implanted 23 and 32 mm passive integrated transponder (PIT) tags on survival, growth, and body condition of juvenile Atlantic salmon *Salmo salar*. Rate of tag retention and healing of the tagging incision were also evaluated. Atlantic salmon of three different size classes (I: 80 to 99 mm fork length (FL), II: 100 to 119 mm FL, III: 120 to 135 mm FL) were allocated to each of five experimental treatment groups: control, sham-operated (surgery without PIT-tag implantation), 23 mm PIT-tag implantation with and without suture closure of the incision, and 32 mm PIT-tag implantation without suture closure.

Results: Over the 35-day experiment, mortality occurred only among fish tagged with 32 mm PIT tags (14%) and all fish larger than 103 mm FL survived. Non-sutured Atlantic salmon between 80 and 99 mm FL implanted with 23 mm PIT tags had a significantly lower mean specific growth rate of mass compared with untagged (control and sham-operated) and sutured conspecifics. However, no significant difference in growth was found between untagged fish and 23 mm PIT-tagged fish 100 to 135 mm FL. Implantation of 32 mm PIT tags decreased growth in all size classes. Regardless of size class, body condition of the fish was not affected by PIT tagging. Retention rates of 23 mm PIT tags with and without suture closure were 100% and 97%, respectively; retention of 32 mm PIT tags without suture closure was 69%. At the end of the experiment, tagging incisions without suture closure were generally well-healed. Fungal infection and inflammation around the incision site occurred only when suture was used, in 46% of size class I, 21% of size class II and 38% of size class III.

Conclusions: Although suture closure of the incision following 23 mm PIT-tag implantation had a positive impact on growth of fish smaller than 100 mm FL, we advise against the use of sutures due to high rates of fungal infection around the incision site. Hence, results suggest that surgical implantation of 23 mm PIT tags without suture closure of the incision is a feasible method for marking juvenile Atlantic salmon 100 to 135 mm FL. Further, we caution researchers about the use of 32 mm PIT tags in juvenile Atlantic salmon 80 to 135 mm FL due to high rate of tag rejection and reduced survival and growth.

Keywords: Biotelemetry, PIT tagging, Salmonids, Surgery, Tagging effect

Background

Over the past decades, passive integrated transponder (PIT) tags have proven to be a powerful tool for monitoring migration, growth, survival, and spatio-temporal distribution of various fish species [1-6]. PIT tags are advantageous due to their longevity, small size, and ability to equip individuals with a unique identification code. Furthermore, PIT tags provide a cost-effective and easy internal tagging technique for gathering information about fish ecology. Currently, commercially available PIT tags typically vary in length from 11 to 32 mm. Smaller tags generally have a lower detection range than larger ones when energized by external antennae via an electromagnetic signal [7]. As a consequence, the use of smaller transponder tags (for example, 11 to 12 mm) has largely been restricted to laboratory applications and field studies in systems with water depths less than 40 cm [8,9]. Larger tag sizes, such as 23 or 32 mm, offer a detection range up to 100 cm, extending their applicability for certain

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telemetry applications [10,11]. However, because intracoelomic implantation of PIT tags may adversely affect fish [12,13], scientists must balance the often conflicting needs of high detection range and low tag burden (tag size relative to fish body size), especially in telemetry studies involving smaller-bodied fish.

A growing number of studies have addressed the adverse effects of PIT tagging on smaller fish and/or various life stages [12-18]. In juvenile salmonids, the impact of smaller PIT tags (11 to 12 mm) on growth and mortality have generally been negligible (for example, brown trout *Salmo trutta* [3]; Atlantic salmon *Salmo salar* [19]) and tag retention rates are usually high (that is, 97% to 100% [3,19,20], see [21] for an exception). The efficacy of 23 mm PIT tags has also been tested among different species of juvenile salmonids (Atlantic salmon *S. salar* [10,22]; coho salmon *Oncorhynchus kisutch* [20]; steelhead *O. mykiss* [14,20,23]). For instance, Bateman and Gresswell [14] examined growth and survival of steelhead (73 to 97 mm FL) following intracoelomic surgical implantation of 23 mm tags. The authors found transient reduction in growth and higher mortality among tagged steelhead compared to control and sham-operated fish (that is, fish that received surgery but no PIT tag). Using the same-sized tag and surgical technique, Roussel *et al.* [10] used PIT technology to study diel movements and habitat use by Atlantic salmon parr 64 to 94 mm FL. However, no control or sham-operated group was included in the study, sample size was small (n = 33), and growth was not evaluated as an endpoint. Thus, there is a need for additional evaluation and clarification of whether and to what extent 23 mm PIT tags influence survival and growth of juvenile Atlantic salmon. Moreover, to our knowledge, no studies have previously tested the efficacy of 32 mm PIT tags in smaller fish. Hence, the aim of the present study was to elucidate the potential effects of surgically implanted 23 and 32 mm PIT tags on survival, growth, and body condition of juvenile Atlantic salmon. Incision healing with and without suture closure and rate of tag retention were also assessed.

Results

Mortality

While there were no mortalities for control, sham-operated, and 23 mm PIT-tagged fish with and without suture closure of the tagging incision, 10 fish with 32 mm PIT tags died during the laboratory experiment. This mortality rate (14%) was statistically different from that of the other treatments (Chi-square test, $\chi^2_4 = 41.143$, $P < 0.0001$). The majority of mortalities (90%) resulting from 32 mm PIT tags occurred within 11 days after tagging (the last fish died 21 days after tagging) but all Atlantic salmon larger than 103 mm FL survived. However, there were no mortalities during surgery or the recovery period immediately after surgery and tag insertion. Average tag-to-body mass ratio of dead and surviving fish tagged with 32 mm PIT tags were 11.1% and 5.0%, respectively. External and internal examination of the dead fish revealed no abnormalities (for example, accidental cuts or punctures to internal organs from the scalpel, tissue inflammation, or infection around incision).

Growth rate

At the end of the 35-day experiment, the mean specific growth rate (SGR; g day^{-1}) differed significantly among treatments within each of the three size classes (one-way analysis of variance (ANOVA), degrees of freedom = 96 to 114, $F \geq 4.204$, $P \leq 0.004$; Figure 1). For size class I (80 to 99 mm FL), post hoc comparisons showed that fish tagged with 23 and 32 mm PIT tags without suture closure of the incision had significantly lower mean SGR than control, sham-operated, and 23 mm PIT-tagged individuals with suture closure (Tukey honestly significant difference (HSD) test, $P \leq 0.041$; Figure 1). In fact, the mean SGR was negative for non-sutured fish implanted with 23 and 32 mm PIT tags at the end of the study. For size classes II (100 to 119 mm FL) and III (120 to 135 mm FL), only growth of fish implanted with 32 mm tags differed significantly from that of the other treatments (Tukey HSD test, all $P \leq 0.001$; Figure 1). No differences in mean SGR was found between control and sham-operated fish within the three size classes (Tukey HSD test, all $P \geq 0.990$).

Body condition

Including all size classes, a full-factorial analysis of covariance on the final body mass of the Atlantic salmon with treatment as fixed factor and final length as covariate demonstrated no interaction between treatments and final length ($F_{4,\ 322} = 1.961$, $P = 0.100$). A subsequent additive model including the interaction term in the error variance showed a significant effect of final length ($F_{1,\ 326} = 31587.767$, $P < 0.0001$), but no effect of treatment ($F_{4,\ 326} = 0.714$, $P = 0.583$). It seems, therefore, that the body condition of the Atlantic salmon was not affected by surgical implantation of 23 and 32 mm PIT tags during the study.

Tag retention and incision healing

The tag retention rates without suture closure were 97% and 69% for the 23 and 32 mm PIT-tagged treatment group, respectively, and no tag loss was recorded when sutures were used. Regardless of tag size, the majority of tag losses (88%) occurred within the first 16 days of the experiment and no 23 mm PIT tags were lost after day 13. Fish that lost their 32 mm PIT tag were on average significantly smaller (96 ±1.88 mm FL, n = 22) than those that retained their tag (114 ±2.98 mm FL, n = 50) (t-test, $t_{70} = 4.863$, $P < 0.0001$). The two fish that rejected their 23 mm PIT tag measured 82 and 86 mm FL at tagging.

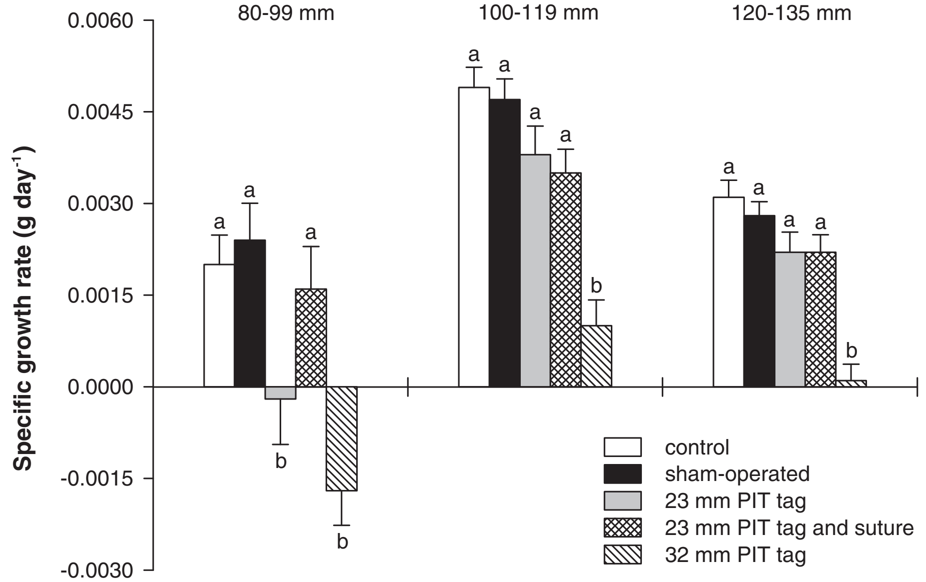

Figure 1 Mean specific growth rate of Atlantic salmon *Salmo salar* among treatment groups in three different size classes. Data calculated as mean specific growth rate (g day^{-1}) ± standard error. Sample size for size classes I (80 to 99 mm FL), II (100 to 119 mm FL) and III (120 to 135 mm FL) were as follows: control (n = 24, 24, 24), sham-operated (n = 24, 24, 24), 23 mm PIT tag (n = 22, 24, 24), 23 mm PIT tag and suture (n = 24, 24, 24), 32 mm PIT tag (n = 7, 16, 23). Differences in sample size are due to mortality and loss of PIT tags. Within each size class, bars not sharing the same letter are significantly different at P <0.05 (Tukey honestly significant difference). PIT, passive integrated transponder.

The tagging incisions without suture closure (n = 216) were generally well healed and no signs of tissue inflammation or infection were observed. By contrast, 31% (n = 22 of 72) of sutured incisions exhibited mild to moderate fungal infection around the incision and suture insertion site. Furthermore, a few sutured incisions (4%) were partially open and highly inflamed and infected, resulting in an overall infection rate of 35%. Although the prevalence of fungal infection was particularly high for size class I (46%), no significant difference in the infection rate was observed between the three size classes (Chi-square test, $\chi^2_2 = 2.288$, $P = 0.319$). The prevalence of fungal infection was 21% and 38% for size class II and III, respectively. It is also noteworthy that all fish that lost the suture (15% shed rate) during the experiment had well healed incisions free of infections.

Discussion

Mortality

During the 35-day laboratory experiment, 10 fish tagged with 32 mm PIT tags died (14%), whereas no mortalities occurred among control, sham-operated or 23 mm PIT-tagged fish with and without suture closure of the incision. The majority of mortality (80%) occurred in fish 80 to 84 mm FL. This is in agreement with results from other studies that typically report higher mortalities in smaller individuals following tagging [18,21,24,25]. For example, Acolas *et al.* [21] demonstrated that increases in length of juvenile brown trout (41 to 70 mm FL) significantly enhanced the probability of survival after implantation of 11.5 mm PIT tags. Similar results have been reported for Atlantic salmon parr (60 to 69 mm FL) using 11.5 mm PIT tags [24].

The exact cause of death in 32 mm PIT-tagged fish is somewhat unclear. Necropsy revealed no evidence of cuts or punctures to internal organs from the scalpel and epidermis infection was not observed around incisions. However, as the peritoneal cavity of the smaller fish was very limited in its capacity to accommodate a 32 mm tag, it is possible that the tag interfered with vital body organs. Furthermore, direct observations during the study revealed that implanted 32 mm PIT tags influenced buoyancy of tagged fish and resulted in unnatural swimming behavior. This may suggest that the swim bladder was not able to compensate for the additional tag weight. Because a substantial part of the body cavity was occupied by the 32 mm PIT tag, it is also possible that there was not enough room for full expansion of the swim bladder.

In a previous laboratory study, Roussel *et al.* [10] reported a mortality rate of 21% for Atlantic salmon parr (<84 mm FL) following surgical implantation of 23 mm PIT tags. However, the authors did not compare the survival of tagged fish with a control group and sample size was small (n = 33). A 99% survival rate for Atlantic salmon (>90 mm FL) tagged with 23 mm PIT tags was reported by Zydlewski *et al.* [22]. Hence, our results and those of

Zydlewski *et al.* [22] suggest that 23 mm PIT tags have no measurable impact on survival of juvenile Atlantic salmon 80 to 135 mm FL.

Growth and body condition

While non-sutured fish between 80 and 99 mm FL implanted with 23 mm PIT tags had a lower mean SGR compared to control and sham-operated individuals, no significant difference was detected in fish with sutured incisions. Although the reason remains unknown, this finding suggests that incision suturing following tagging may have a positive impact on the mean SGR of Atlantic salmon 80 to 99 mm FL. It should be noted, however, that the average growth of sutured fish between 80 and 99 mm FL was still somewhat lower (16%) than growth of the control group and 46% of the fish exhibited fungal infection around the incision site. Hence, long-term studies investigating the effect of fungal infection on growth and survival are needed before recommending the use of suture in Atlantic salmon 80 to 99 mm FL. The mean SGR for fish between 100 and 135 mm FL was not significantly affected by 23 mm PIT-tag implantation with and without suture closure of the incision, although the mean SGR was up to 30% lower compared to the control group. However, 32 mm PIT tags significantly affected mean SGR of all size classes. Mean SGR was not significantly different between control and sham-operated fish within all size classes, implying that the surgical procedures had no effects on mean SGR *per se*, which is consistent with earlier findings [14,26,27]. Therefore, the presence of the tag is evidently responsible for the decreased growth, perhaps due to increased energy expenditure of carrying the tag or physiological adjustments needed to accommodate it.

Several other studies have reported depressed growth after tagging, but there is much variation in the extent and duration of this impact, depending on factors such as species, size, and environmental conditions [12,14,18,21,24]. Bateman and Gresswell [14] showed decreased growth in juvenile steelhead (73 to 97 mm FL) during the first 20 days after surgical implantation of a 23 mm PIT tag. However, this was compensated for 30 days after tagging by increased growth of the tagged fish. Similar results have been found for juvenile brown trout (41 to 70 mm FL) and Atlantic salmon (60 to 69 mm FL) up to 60 days after implantation with 11.5 mm tags [21,24]. Tatara [13] demonstrated that the probability of experiencing positive growth in steelhead parr (45 to 96 mm FL) implanted with 12 mm PIT tags was size-dependent, and it was concluded that length at implantation should be above 74 mm FL to avoid negative growth. While we were not able to identify the length at tagging that would prevent negative effect of 32 mm tags on growth of Atlantic salmon, results indicate that implantation of 23 mm PIT tags has no significant effect on growth of fish 100 to 135 mm FL. As the duration of the study was short (35 days), it remains unknown whether the somewhat lower growth of the PIT-tagged fish would eventually be accounted for through compensatory growth. Moreover, although the condition of the fish in terms of length-mass relationships was similar among treatments at the end of the experiment, slower growth rates due to tagging could have long-term repercussions on body condition. Additional studies are needed to examine the long-term impact of 23 and 32 mm PIT tags on growth and body condition of Atlantic salmon.

Tag retention and incision healing

Tag retention of 23 mm PIT tags was high (97%) when the tagging incisions were left to heal without suture closure and no tag loss occurred when sutures were used. The two fish that lost their 23 mm tags were both below 90 mm FL. These results are in accordance with those of Zydlewski *et al.* [22], reporting a 23 mm PIT tag retention rate of 99% for Atlantic salmon (>90 mm FL). Roussel *et al.* [10], however, found a tag loss rate of 15% for Atlantic salmon parr (64 to 94 mm FL) implanted with the same-sized tag, but 100% retention when suture was used to close the incision. Suturing may therefore considerably lower the rate of tag loss through the incision in smaller fish [10,12,25]. Additionally, suture closure has been shown to enhance the rate of incision healing and reduce the risk of infection and epidermis inflammation for certain fish species [12]. In the current investigation, however, tissue inflammation and fungal infection were only observed when sutures were used and non-sutured incisions were generally well-healed. This observation and low 23 mm PIT tag rejection rate without suture closure, suggest that PIT tag incisions should not be closed with suture in juvenile Atlantic salmon 80 to 135 mm FL. At the same time, suturing the incision increases handling time and risk of puncturing internal organs [12]. It should be emphasized, that tagging incisions should be made small enough (3 to 4 mm) so that the 23 mm transponder tag cannot easily work its way out of the wound again. Finally, tissue adhesives (usually cyanoacrylate) are occasionally used as an alternative to sutures, as it might be a faster and less intrusive way of closing incisions [28,29]. However, because incisions closed with adhesives sometimes reopen and result in tissue inflammation, the efficacy of this closing technique may be questioned [25,29,30]. Clearly there is a need for additional work focused on comparing multiple incision closure methods in smaller fish.

After 35 days, 31% of the Atlantic salmon had lost their 32 mm transponder tag. Such a high tag loss rate is greater than the accepted value for juvenile salmonids, which is usually below 10% [3,14,19,20,22,23]. The majority of 32 mm tag loss occurred among smaller individuals and was most likely caused by limited body cavity capacity. In a narrow peritoneal cavity, pressure against the tag could cause it to be pushed through the tagging incision. Other researchers have also reported a relationship between length at tagging and rate of tag retention, with larger individuals typically having the highest rate of tag retention [21]. Most tag losses occurred within the first half of the experiment and tag loss presumably decreased as tagging incisions began to heal. Thus, additional tag losses related to tagging are believed to be negligible 35 days after surgery [14,25].

Conclusions

Surgical implantation of 23 mm PIT tags into the body cavity of juvenile Atlantic salmon (80 to 135 mm FL) did not affect survival or body condition and tag retention was high with and without absorbable suture closure of the tagging incisions. Although suture closure of the incision had a positive effect on growth of the smaller 23 mm PIT-tagged fish (80 to 99 mm FL), 46% of the incisions were infected by fungus. Fungal infections were also observed in sutured fish 100 to 119 mm FL (21%) and 120 to 135 mm FL (38%). By contrast, all non-sutured incisions were generally well-healed and no signs of epidermis inflammation or fungal infection were observed. Hence, at this point, we caution researchers about the use of sutures in juvenile Atlantic salmon 80 to 135 mm FL. Further studies are needed to examine the long-term effects of fungal infection around the incision and suture site on growth and mortality of juvenile Atlantic salmon.

When leaving the incision non-sutured, results indicate that 23 mm PIT tags have no adverse effect on growth of Atlantic salmon 100 to 135 mm FL. We conclude that intracoelomic implantation of 23 mm PIT tags without suture closure is a useful method for individual marking of Atlantic salmon 100 to 135 mm FL. However, in studies where growth is not a parameter of interest, 23 mm PIT tags may be useful in Atlantic salmon as small as 80 mm FL. Finally, we recommend that 32 mm PIT tags should not be used use in juvenile Atlantic salmon (80 to 135 mm FL) due to high mortality, high tag loss rate, and reduced growth. We advocate studies on larger Atlantic salmon to establish a suitable size limit for using these tags.

It is difficult to anticipate whether the results from the present laboratory study will be applicable in natural systems. Fish in a hatchery are not exposed to the same stressors (predation, food, density, pathogens) that fish in the wild experience. As such, future tagging studies would benefit greatly from combining both laboratory and field experiments. Moreover, it is possible that the effect of tagging hatchery-raised fish may be different from those of wild fish. Nevertheless, the results of the present investigation should be useful for managers and scientists in monitoring the biology and ecology of Atlantic salmon.

Methods

Experimental fish

Fish used in the experiment were hatchery-reared offspring of wild Atlantic salmon caught by electrofishing in River Storå, Denmark. Fertilized eggs were incubated in egg trays and hatched in late March 2011 at the Danish Centre for Wild Salmon (DCV) in Randers, Denmark. After hatching, the alevins were maintained in the hatching trays until the yolk sac was completely absorbed. Fish were then transferred to flow-through tanks for exogenous feeding and kept under ambient photoperiod and temperature (4°C to 17°C) conditions. The Atlantic salmon were fed daily with commercial trout pellets equivalent to 1.5% to 4% of body mass. All study fish were handled in accordance to the guidelines described in permission (2012-DY-2934-00007) from the Danish Experimental Animal Committee.

Experimental protocol

The laboratory experiment was conducted at the hatchery facilities at DCV from 21 December 2011 to 25 January 2012. A total of 360 Atlantic salmon were divided into three different size classes (n = 120 per size class): I: 80 to 99 mm FL, II: 100 to 119 mm FL, III: 120 to 135 mm FL. Within each size class, fish were randomly assigned to one of five treatment groups: control, sham-operated, 23 mm PIT tag, 23 mm PIT tag and suture, and 32 mm PIT tag. This resulted in 24 fish from each size class per treatment. These fish were evenly and haphazardly distributed among six experimental tanks. Hence, each tank contained fish from all five treatments and each treatment group consisted of four fish from each size class per tank. The net result was 60 fish in each tank (that is, 12 fish per treatment group). Within size classes, there were no significant differences in length and mass among the treatment groups at the beginning of the experiment (one-way ANOVA, $F_{4,115} \leq 2.236$, all $P \geq 0.069$).

Treatment fish were placed in an anesthetic bath (benzocaine 20 mg L^{-1}) until the opercular rate became slow and irregular (4 to 5 min). Once unresponsive, the fork length and body mass were measured to the nearest 1 mm and 0.1 g, respectively. Sham-operated fish received a 3 to 4 mm ventrolateral incision, 5 to 7 mm anterior to the muscle bed of the pelvic fins on the left side of the body. Atlantic salmon in the 23 mm PIT-tagged group were treated similarly except a uniquely coded 23 mm PIT tag (RI-TRP-RRHP, half duplex, 134 kHz, diameter 3.85 mm and weight 0.6 g in air; Texas Instruments, Plano, Texas, USA) was inserted into the peritoneal cavity through the incision. For the 23 mm PIT-tagged fish with suture closure treatment, incisions were closed with one stitch of absorbable suture (Vicryl 5–0 FS-2; Ethicon, Piscataway, NJ, USA) tied with a single surgeons knot. Fish in the 32 mm PIT-tagged treatment group were subjected to a 3 to 4 mm ventrolateral incision posterior to the pelvic fins. A 32 mm PIT tag (RI-TRP-WR2B, half duplex, 134 kHz, diameter 3.85 mm and weight 0.8 g in air; Texas Instruments) was gently pushed anteriorly into the body cavity and the incision was left to heal without suture closure. Control fish were handled in the same manner as fish in the other treatments but no surgery was performed and no tag was implanted. Sham-operated fish were included in the experiment to isolate the effects of the surgery procedures from the effects of the PIT tags. The initial mean tag-to-body mass ratio in air was 5.1% (range: 2.2% to 13.6%) for Atlantic salmon tagged with 23 mm PIT tags and 6.7% (range: 2.7% to 14.8%) for those tagged with 32 mm PIT tags.

All control and sham-operated fish received a unique dye-mark combination on the left and/or right side of the caudal peduncle using a Panjet inoculator to allow for individual recognition during the experiment. Fish were marked with Alcian Blue, Irgafin Red P or a combination of the colors and the maximum number of dye-marks per fish was three. When used properly, jet injection of dye has no measurable effect on survival and growth of juvenile Atlantic salmon [31-33]. However, to ensure that the potential impacts of PIT tagging on mortality and growth were entirely isolated from any negative effects stemming from color marking, all PIT-tagged fish were also given dye-marks on the caudal peduncle region. Excess dye was carefully flushed off the fish with water as recommended by Hart and Pitcher [34]. The duration of the procedures for the control, sham-operated, 23 mm PIT tag, 23 mm PIT tag with suture closure, and 32 mm PIT tag treatment groups took on average 33, 38, 43, 77 and 44 s, respectively. Fish were not fed within 24 hours of surgery and the same surgeon performed all surgeries. All surgery equipment was disinfected (96% ethanol) prior to use and scalpel blades were changed frequently to avoid tearing of the tissue.

After treatment, fish were placed in an aerated barrel (60 L) until they recovered from anesthesia (3 to 4 min) and then transferred to the experimental tanks. The tanks (200 × 200 cm) were supplied with fresh water from a flow-through filtration system at a flow rate of 30 L min^{-1} ensuring well-oxygenated water. The water depth was adjusted to 35 cm, resulting in a water volume of 1,400 L. The average water temperature in the tanks was 6.9°C (range: 5.5°C to 7.5°C) and the photoperiod followed natural day-light cycles. Fish were fed during light hours with commercial trout pellets (Aller Performa, Aller Aqua, Denmark) at a ratio of 2% body mass per day using automatic feeders. The tanks were inspected daily for dead fish and rejected PIT tags. Dead fish were removed from the tanks, individually identified, measured, weighed, and examined externally and internally. The codes of rejected PIT tags found at the bottom of the tanks were recorded using a handheld reader (Agrident GmbH, APR350, Barsinghausen, Germany). After 35 days, the surviving Atlantic salmon were killed with an overdose of benzocaine, measured, weighed, and identified. The weights of fish tagged with 23 and 32 mm PIT tags were corrected by subtracting the weight of the tag from the final body mass (0.6 g and 0.8 g, respectively). The tagging incisions were inspected for infection, inflammation, and healing.

Data analysis

Fish that died or lost their PIT tag during the experiment were only used in analyses of survival and rates of tag retention. The percent tag loss was calculated as number of lost tags divided by the total number of fish tagged multiplied by 100. At the end of the experiment, the survival rate was calculated and a Chi-square test was used to compare survival among treatment groups. Tag-to-body mass ratio (%) in air was calculated with the formula:

$$\text{Tag–to–body mass ratio} = \left(\text{tag weight} * M_i^{-1}\right) * 100\%,$$

where M_i is the mass of fish prior to tagging.

The SGR (g day^{-1}) was calculated for each individual fish according to the following equation:

$$\text{SGR} = \left(\log_e M_f - \log_e M_i\right) * t^{-1},$$

where M_i and M_f are the initial and final mass (g), respectively, and t is the time in days. A one-way ANOVA was used to determine if there were differences in SGR among size classes of treatment groups. Tukey HSD multiple comparisons were subsequently performed to determine which treatment groups had significantly different SGR among size classes. Tank number was included as a random effect variable in the analyses.

An analysis of covariance was conducted to elucidate the effect of experimental treatments, length, and the interaction between these two variables on the body mass of the Atlantic salmon at the end of the experiment. The fish length-mass relationship was used as a proxy for body condition. Analyses were carried out on log-transformed length-mass data.

All statistical analyses were performed in SPSS 20.0 (Statistical Package for the Social Sciences; SPSS Inc, Chicago, IL, USA). Prior to analysis, data exploration was carried out as per Zuur *et al.* [35]. Assumption of homogeneity of variance and normal distribution for the models were ensured by visual inspection of residual plots. Variation in association with recorded mean values is given as standard error throughout. Statistical significance for all analyses was set at $\alpha = 0.05$.

Abbreviations

ANOVA: Analysis of variance; DCV: Danish Center for Wild Salmon; FL: Fork length; HSD: Honestly significant difference; PIT: Passive integrated transponder; SGR: Specific growth rate.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MHL, ANT and KA designed the experiment. MHL and ANT carried out the experiment and analyzed the data. All authors contributed to draft the manuscript, and read and approved the final manuscript.

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