Charles University in Prague, Faculty of Science
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Age structure and growth of wild brown trout in relation to population density and habitat quality

Ph.D. Thesis

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

Declaration:

I declare that this thesis has been fully worked out by me and all of the sources and literature were cited. Neither this thesis nor any of the publications attached within have been submitted for the purpose of obtaining the title of Ph.D. or any other title at another institution.

In Prague, 07.07.2015

Signature:

I declare that Libor Závorka as a co-author of the article „Eggs from anadromous adults provide marine-derived nutrients to Atlantic salmon and brown trout parr prior to the onset of winter – observations from a Swedish coastal stream” had significantly participated in manuscript preparation.

In Göteborg, 07.07.2015, Joacim Näslund

Abstract

Brown trout *Salmo trutta L.* is a fish species with high socio-economic value, which is favourable among anglers and a successful invader worldwide. The aim of this thesis is to explore environmental factors affecting body growth and survival of brown trout with emphasis on density dependent selection in juvenile life stages. This thesis is specifically focused on: (1) effect of population density on growth and survival with respect to a dynamic of a local group of individuals (papers **I** and **II**); (2) effect of inter-individual differences in behaviour on the relationship between individuals life-history traits and available resources (papers **III** and **IV**); (3) link between demo-genetic structure of population and growth and mortality rates of individuals (papers **V**, **VI** and **VII**).

Datasets for this thesis were collected during a long-term mark-recapture study on wild brown trout population (2005-2011) in the catchment of the Otava River in Šumava National Park (Czech Republic) and a set of field and laboratory studies conducted on wild populations in streams on west coast of Sweden.

In accordance with some previous studies, this thesis showed that growth of juvenile brown trout is negatively affected by population density. Nonetheless, this thesis reveals that the negative effect of density dependent growth can be mitigated by familiarity with environment and towards conspecifics within a local group. This thesis also demonstrates that consistent inter-individual differences in activity can be linked to differences in growth rate, mortality and dispersal. The consistent behavioural types (animal personalities) described here, affect the way in which individuals utilize resources and they can, for example, affect propensity for egg eating during autumn spawning season. This thesis shows, with an example of a small migratory connected mountain watershed that differences in growth and survival rate associate with a demo-genetic structure of subpopulations within a metapopulation.

Relationships between behaviour, environment and life-history traits reported in this study were tested on several populations of brown trout in Europe, and they highlight the importance of local adaptations for productivity of brown trout populations. Specific findings of this thesis can be applied in management and conservation of populations of brown trout but also other species of stream dwelling freshwater fish.

Abstrakt

Pstruh obecný *Salmo trutta* L. je hospodářsky významná lososovitá ryba, která zároveň patří k nejúspěšnějším invazním druhům sladkovodních ryb na světě. Cílem této disertační práce bylo studium parametrů ovlivňujících rychlost růstu a přežívání jedinců tohoto druhu s důrazem na vliv populační hustoty na juvenilní stádia. Konkrétně je tato práce zaměřena na: (1) vliv hustoty populace na růst a přežívání jedinců s ohledem na dynamiku lokálních skupin jedinců (publikace **I** a **II**), (2) vliv rozdílného chování jedinců na vztah mezi růstem a přežíváním jedince v prostředí s variabilním rozložením zdrojů (publikace **III** a **IV**), (3) vztah mezi rychlostí růstu, mortalitou a demo-genetickou strukturou populace (publikace **V**, **VI** a **VII**).

Údaje pro tuto práci pochází z dlouhodobého sledování populace pstruha obecného (2005 - 2011) v povodí řek Vydra a Křemelná v Národním Parku Šumava a kombinace terénních a laboratorních experimentů s divokými populacemi z toků na západním pobřeží Švédka.

V souladu s přechozími studiemi tato práce ukazuje, že růst juvenilních jedinců je negativně ovlivněn populační hustotou. Nicméně negativní vliv početnosti může dle výsledků této práce být výrazně omezen známostí habitatu a ostatních jedinců ve skupině. Dále tato práce ukazuje, že i v rámci jedné populace pstruha obecného existují permanentní rozdíly v aktivitě, které jsou spojené s rychlostí růstu, pravděpodobností přežití a migračním chováním jedince. Zde popsané stálé behaviorální typy (osobnosti zvířat) ovlivňují způsob jaký jedinci využívají habitat nebo jakou mají potravní preferenci (například sklon ke kanibalistické konzumaci jiker). Na příkladu malého migračně propojeného horského povodí tato práce ukazuje, že rozdíly v růstu a mortalitě jedinců jsou spojené s demo-genetickou strukturou subpopulací rámci jedné metapopulace.

Závislosti mezi chováním, prostředím, růstem a mortalitou demonstrované v této práci byly testovány na několika místech v Evropě a zdůrazňují důležitost lokálních adaptací na produktivitu populací pstruha obecního. Jednotlivé výstupy této práce mohou nalézt využití při hospodaření a ochraně populací pstruha obecného, ale také dalších druhů sladkovodních ryb.

LIST OF PUBLICATIONS AND MANUSCRIPTS INCLUDED IN THE PH.D. THESIS

I **Závorka L.**, Horký P., Höjesjö J. and Slavík O. (2015) Effect of individuals' local persistence, spatial and temporal scale on density-dependent growth: a study in brown trout. Ethology, Ecology and Evolution (IF 1.150)

II **Závorka L.,** Näslund J., Aldvén D., Höjesjö J. and Johnsson J. Familiarity mitigates effects of density dependent competition: an experimental study on territorial salmonid fish. (Submitted to Ethology in May 2015, IF 1.556)

III Näslund J., Aldvén D. and **Závorka L.** (2015) Eggs from anadromous adults provide marine-derived nutrients to Atlantic salmon and brown trout parr prior to the onset of winter – observations from a Swedish coastal stream. Environmental Biology of Fishes (IF 1.356)

IV **Závorka L.**, Aldvén D., Näslund J., Höjesjö J. and Johnsson J. (2015) The fluctuating cost of high activity – an extension of the Pace of Life Syndrom hypothesis. Behavioral Ecology 26: 877-884 (IF 3.157)

V **Závorka L.**, Slavík O. and Horký P. (2014) Validation of scale-reading estimates of age and growth in a brown trout *Salmo trutta* population. Biologia 69, 691-695. (IF 0.696)

VI **Závorka L.**, Slavík O. and Horký P. (2013) Individual growth and population distribution of brown trout in pristine headwaters. Central European Journal of Biology 8, 263-271. (IF 0.633)

VII **Závorka L.**, Horký P., Kohout J., Kalous L. and Slavík O. (2015) Demogenetic structure of brown trout *Salmo trutta* Linnaeus, 1758 populations in mountain headwaters: implications for conservation management. Journal of Applied Ichthyology 31, 501-508. (IF 0.903)

Content

Introduction

Negative feedback between population density and population growth rate at densities close to the carrying capacity of the environment is the basic biological rule governing size of populations in nature. This negative feedback is a consequence of competition among individuals for thinning resources such as food, shelter or mates (Gotelli, 2008). The essential element here is the competition among individuals either through direct aggressive interaction or depletion of resources, which become unavailable for the other members of a population (Davies et al., 2012). Inter-individual interactions are not only affected by the ambient environment, but also by characteristics of each contesting individual (Biro and Stamps, 2008; Adriaenssens and Johnsson, 2009). Self-thinning in animal populations has been extensively studied and brown trout is a common model of these studies (Elliott, 1994; Lobón-Cerviá, 2012; Bohlin et al., 2002; Kaspersson and Höjesjö, 2009); however, the mechanism of inter-individual behavioural interactions behind this process has received less attention.

An example, which is elaborated in this thesis, is the negative effect of population density on individual body growth. The decrease of body growth can, during its early stage, buffer the effect of increased population density, because the smaller individual consumes fewer resources and thus there can be higher abundance of them (Lobón-Cerviá, 2007). Nonetheless, the decreased growth rate indicates poor fitness of individuals caused by increased competition stress (Scott, 1988; Harrison et al., 2011) and it eventually leads to higher mortality, lower fecundity and decrease of population growth rate (Elliott, 1994; Gotelli, 2008). Body size is a trait which, especially in animals with undetermined growth, correlates with biological fitness (Stearns, 1973). Large individuals have higher fecundity and probability of survival and they are more successful in territorial conflicts (Jonsson et al., 1991; Scott, 1994; Fordham et al., 2007; Johnsson et al., 1999). However, growth rate is not affected only by individuals' abundance, as habitat characteristics and the behaviour of the individual also has a significant effect (Biro and Stamps, 2008; Davies et al., 2012). This thesis tested the importance of a local group dynamic, fluctuation of resources and behavioural strategies of individuals on density dependent growth and survival.

Life-history plasticity of brown trout

Brown trout (*Salmo trutta* L.) is a highly plastic species displaying a range of ecological adaptations. However, all life-history types of brown trout have similar initial stage of the life cycle, which usually begins by an autumn spawning of adults in small nursery streams and laying eggs into nests dug by the female in a gravel bed of bottom substrate (Jonsson and Jonsson, 2011). Yolk sac alevins hatch from eggs in early spring and stay hidden in gravel until the yolk sac is absorbed. Fry without the yolk sacs emerge from the gravel and start with active feeding at the end of spring (Jonsson and Jonsson, 2011). First differentiation in individual strategies associated with the life-history types is usually reported after the emergence, but the most distinctive differences occurs at the end of juvenile stage between the first and third year of life (Klemetsen et al., 2003; Jonsson and Jonsson, 2011). There are 3 basic life-histories associated with ontogenetic habitat shift during this period: stream dwelling, lake migratory, and sea migratory (Klemetsen et al., 2003). Even within a life-history type, a range of strategies exist, which are linked to local adaptations of individuals. For example, the sea migration in anadromous populations of brown trout usually occurs between the second and third year of life (Bohlin et al., 1993), however populations from Gotland island in the Baltic sea undertake anadromous migration to brackish water only three months after the emergence (Limburg et al., 2001). Different strategies can be observed also within a population, an example is the strategy of sneaky males (Dellefors and Faremo, 1988). Individuals adopting this strategy display slow growth, early age of maturation and so called sneaky spawning. Sneaky males do not fight for spawning territories same as fully developed males; instead they approach a pair of fully developed mature individuals during the spawning and release sperm to their nest (Dellefors and Faremo, 1988). Life-history strategies evolve together with trade-offs between migration, survival, and growth (Roff, 1992; Hendry, et al. 2004). Elliott (1994) suggests that anadromous populations of brown trout produce abundant juvenile cohorts and juveniles are exposed to strong negative effect of density on growth rate and size dependent survival during a critical period, which lasts until the second summer of life. On the other hand, landlocked populations according to Elliott's observation produce weaker juvenile cohorts where growth and survival of individuals is affected more by abiotic factors than population density. Despite these observations density dependent growth has been reported even in land-locked populations of brown trout (Olsson and Greenberg, 2004).

Social hierarchy and density dependent effect

Brown trout has high fecundity, the same as most fish species, and one female can produce up to several thousands of eggs (Elliott, 1994). Therefore, the abundance of new cohort is usually several times exceeding carrying capacity of a nursery stream (Lobon-Cervia, 2007). Therefore, density dependent processes are assumed to play crucial role in population dynamic of brown trout. Both field and laboratory studies have shown that effect of population density in brown trout is reflected in survival and dispersal of individuals across life stages (Elliott, 1994; Jonsson et al., 1998; Lobón-Cerviá, 2012), but also in the body growth of juveniles (Elliott, 1994; Bohlin et al., 2002; Brännäs et al., 2004; Kaspersson and Höjesjö, 2009).

Brown trout is, especially in juvenile stage, very territorial and recruitment of territory after emergence is essential for their survival (Kalleberg, 1958; Kaspersson and Höjesjö, 2009). Aggressive disputes among juveniles lead to creation of social hierarchy (Kalleberg, 1958), which is appointed by dominance in these agonistic interactions (Abbott et al., 1985). Several studies have reported that familiar groups of juvenile salmonid fishes with stabile social structure experience a decrease of inter-individual aggression (Griffiths et al., 2004; Höjesjö et al., 1998; Seppä et al., 2001). The mitigation of the aggressive interaction among individuals can be gained by temporal (Alanärä et al., 2001) and spatial (Vehanen et al., 1999) dispersion of individuals in feeding habitat, diet shift (Nakano et al., 1999), by using alternative feeding tactic (sneaky feeding – Höjesjö et al., 2005) or by preventing aggressive interaction through signalization of social status by the body coloration (O'Connor et al., 2000). Therefore, it seems to be likely that familiarity among individuals can mitigate negative effect of high population density on growth and survival of juvenile cohorts. However, this hypotheses has never been tested before and it could provide an important insight in to behavioural processes behind density depend effects.

In the natural environment, individuals facing high population density are confronting a choice where they can either stay at the locality at the cost of reduced growth rate and survival, or move to another area with potentially better growth conditions (Einum et al., 2006). However, movement can also induce substantial costs, for example, energetic expenditure and increased risk of predation (Yoder et al., 2004). Increased dispersal and mortality of individuals erodes social structure of local groups, which could consequently increase level of aggressive interactions among individuals (Höjesjö et al.,1998; Slavík et al., 2011). Although dispersal and mortality are ecologically different processes they both cause disappearance of individuals from the population. Both dispersal and mortality are reflected in recapture rate (Kaspersson and Höjesjö, 2009) and thus the recapture rate may help assess persistence of individuals within the locality and group of conspecifics.

Pace-of-life syndrome and density dependent growth rate

Individuals within a population differ in their growth rate even when they have the access to the same amount of food (Davenport and Scott, 1993; Wang et al., 1998; Stamps et al., 1998). There is also a body of studies across species, showing that growth rate of animals is usually lower than their maximal potential to grow (Calow, 1982). Possible explanation of the difference between maximal and actual growth can be that costs of high growth rate outweighs benefits associated with large body (Arendt, 1997; Stamps, 2007). Fast body growth should in theory associate with high metabolic rate and behavioural strategies, which facilitate access to resources, like consistently high aggression, activity, exploration and boldness (Biro and Stamps, 2008, 2010). However, these characteristics should be also related to high risk of injury and mortality or faster aging of organism and its repairing processes (Biro et al., 2004, 2006; Näslund et al., 2015).

The pace-of-life syndrome concept, links the theory described above at several levels of biological organization from metabolic rate to population dynamic (Biro and Stamps, 2008; Réale et al., 2010). The core idea of this hypothesis is that certain traits of metabolism, behaviour, growth, fecundity and survival are linked together and creates the pace-of-life syndrome of an individual (Fig. 1, Réale et al., 2010). For example, an individual with the fast pace-of-life should have high metabolic rate, growth rate, fecundity, and short life span; it should also behave aggressively, actively and boldly, while an individual with slow pace-of-life should have opposite characteristics (Biro and Stamps, 2010; Réale et al., 2010). These consistent inter-individual differences in behaviour create animal personality of an individual (Biro a Stamps 2008; Réale et al., 2010; Adriaenssens a Johnsson 2013). Therefore, individual displaying a personality linked to the fast pace-of-life should have advantage under low population density, when resources are rich and population reaches the highest growth rate, but individuals with slow pace-of-life should be favoured under densities close to the carrying capacity of environment. In this sense the pace-of-life syndrome concept replenishes the older theory of “r” and “K” strategies, which were based on the model of negative feedback between growth rate and abundance in population close to the carrying capacity (Gotelli, 2008).

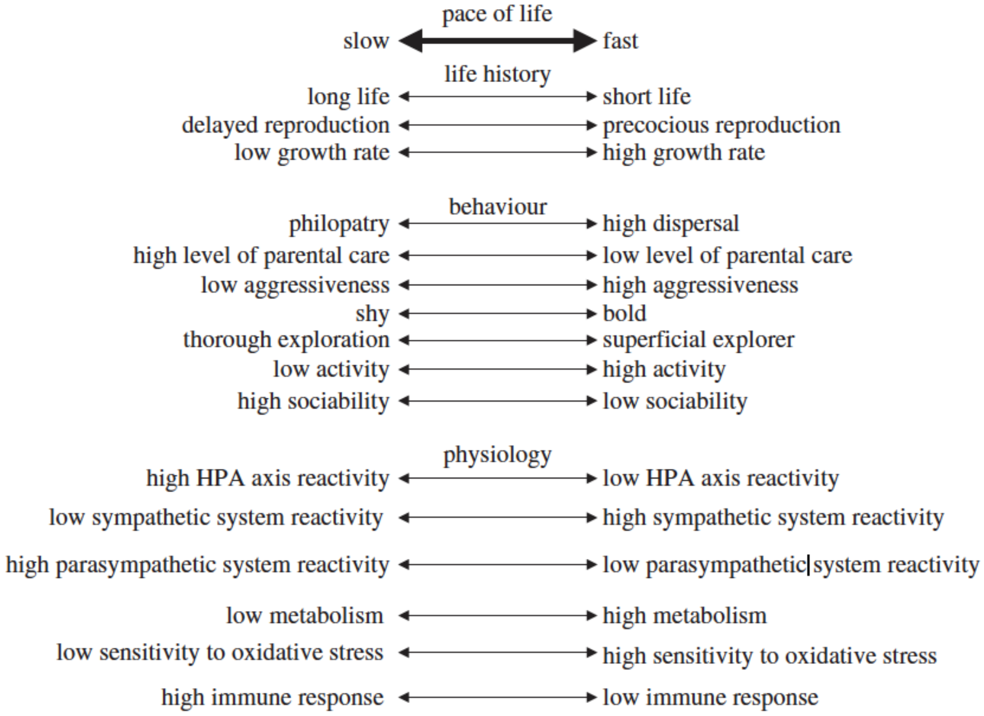

Figure 1 – Association of life-history, physiological and behavioural traits within the pace-of-life syndrome (reprinted from Réale et al. 2010).

Importance of density depend effects for management with brown trout populations

Freshwater ecosystems such as lakes and rivers provide countless services to humanity, and changes in these services could have a large negative impact on our society (Dodds et al., 2013). One of the most important services provided by these ecosystems is to provide fish populations, which are available for recreational anglers and professional fisherman (Beard et al., 2011). Brown trout is the most widely distributed native European salmonid species with significant socio-economical value for its popularity among anglers and high invasive potential outside the European continent (Elliott, 1994; Granek et al., 2008). Despite the history of successful invasions across the globe, European populations of brown trout including Czech Republic have been declining over last 20 years (Jonsson and Jonsson, 2011; Slavík, 2014), yet the causes of this decline are not fully understood.

Populations of stream salmonid fishes display complex demographic and genetic structure (Koizumi, 2011; Kohout et al., 2012), which is linked to local adaptations essential for their viability (Elliott, 1994; Jonsson and Jonsson, 2011).

Therefore, extensive stocking of hatchery-reared individuals is considered one of the major reasons for this decrease (Araki et al., 2008). Stocking of fish from hatcheries can increase competition pressure on wild individuals and has an associated negative effect of density on growth and survival (Bohlin et al., 2002, Lobón-Cervia, 2007). The hatchery environment increases survival of individuals during the critical period, and thus weakens an important selection bottleneck occurring in wild populations (Elliott, 1994; Adriaenssens and Johnsson, 2013). Moreover, a low complexity and high population density in hatchery environment can have negative effect on anti-predation, and social behaviour and learning capacity (Brockmark et al., 2010). Consequently, artificial stocking can introduce individuals with phenotypes and genotypes, which are not adaptive under natural densities. Therefore studying of behavioural adaptations on fluctuating density in wild populations can contribute to a better understanding of the risks associated with artificial stocking of hatchery-raised fish.

Aims of the thesis:

The aim of this thesis is to explore environmental factors affecting body growth and survival of brown trout with emphasis on density dependent selection in juvenile life stages. Studies assembled in this thesis were testing the following assumptions:

(1) Habitat knowledge and familiarity towards conspecifics within a group can facilitate utilization of resources and decrease the level of aggressive interactions among individuals. Therefore, the negative effect of increased population density on growth and survival can be mitigated by familiarity in stabile groups of individuals (papers **I** and **II**),

(2) Growth and survival of an individual is affected by the interaction between individuals personality and availability of resources. Growth and mortality in active individuals is more sensitive to resources availability than in passive individuals (papers **III** and **IV**),

(3) Genetic and demographical isolation among subpopulations of brown trout associates with differences in growth and survival of individuals within a small mountain watershed (papers **V**, **VI** and **VII**).

Methods and material

Studied localities

Data for this thesis originate from several experiments and observations, which were conducted before and during my PhD studies. The first dataset (used in papers **I**, **V**, **VI** and **VII**) comes from a long-term study based on bi-annual sampling and a mark-recapture program performed between years 2005 and 2011. The study was focused on wild population of brown trout in headwaters of the Otava River in Šumava National Park, Czech Republic. Some additional data for paper **V** were collected also in Teplá Vltava River, which is located in the same National Park.

The landscape in the Šumava National Park is mountainous, and the most widespread vegetation type is spruce forest, which alternates with patchily distributed meadows and peat bogs (Fig. 2). The studied headwater streams consist of two main rivers, the Vydra and Křemelná Rivers, which spring at 1,100 m a.s.l. and meet in confluence after ca. 30 km, creating the Otava River. The overall area of the Vydra and Křemelná basins is approximately 224 km2 (Fig. 3). The study streams are cold, oligotrophic and pristine conditions prevail. The substratum of streambeds is heterogeneous and contained sand, gravel, pebbles and boulders, but fine detritus and mud are rare. Fishing is banned and no stocking occurs in study streams. In addition to the brown trout, the studied stream holds populations of bullhead *Cottus gobio*, which is distributed mostly in Křemelná River. Predators of brown trout sighted in the study area are otter *Lutra lutra*, kingfisher *Alcedo atthis*, and burbot *Lota Lota.*

Figure 2 – Example of a typical landscape and vegetation at the sampling sites in NP Šumava

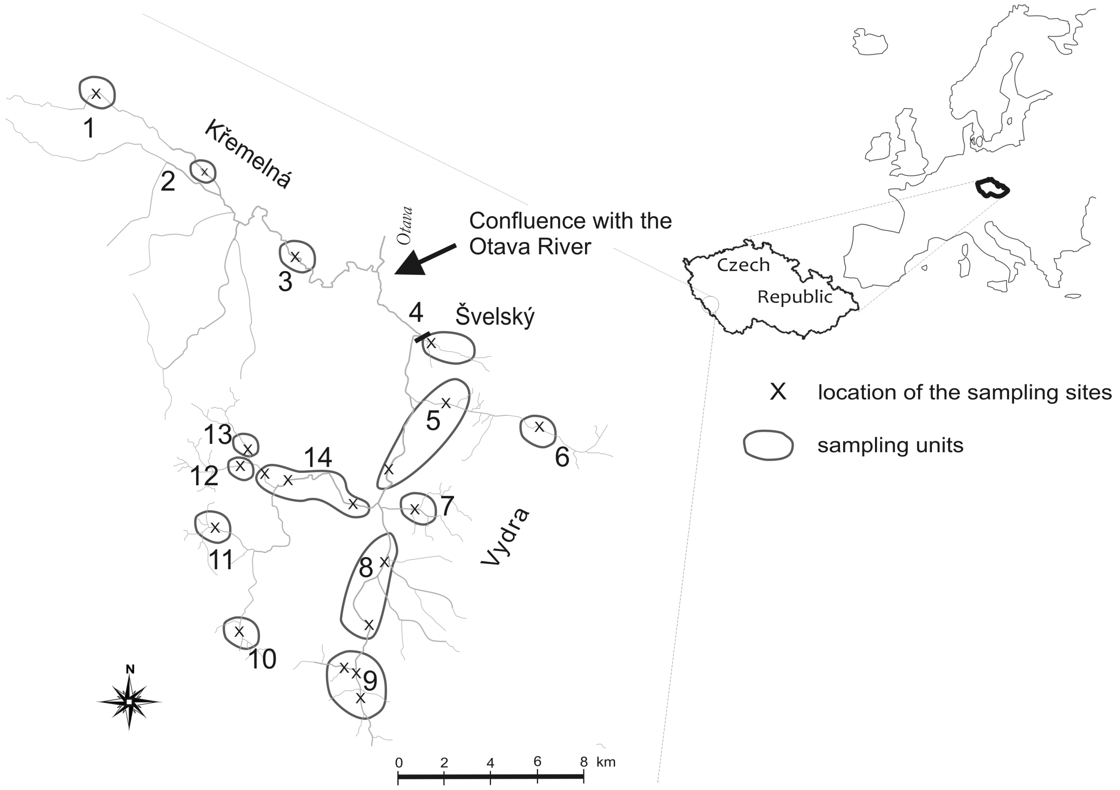

Figure 3 – Map of sampling sites in Šumava NP used for data collection.

Data used in papers **II**, **III** and **IV** were collected on populations of brown trout in two streams (Bodeleån and Jörlandaån) on the west coast of Sweden (Fig. 4). The data sets were collected as three independent studies conducted in years 2013 and 2014.

The nutrient concentrations and conductivity of the Swedish streams are relatively high (compared to the streams in Šumava NP) due to a bedrock composition, which consists of limestone and marine clay and mild oceanic climate, which is influenced by the Gulf Stream. Dense riparian forests, mainly consisting of alder (*Alnus* spp.) and birch (*Betula* spp.) but occasionally also spruce (*Picea* spp.), was shading the experimental streams while the more distant surroundings were dominated by pasture and arable lands (Fig. 5). The streams have stable populations of brown trout, which also is the dominating fish species. However, Atlantic salmon (*Salmo salar*), European minnow (*Phoxinus phoxinus*), stickleback (*Gasteosterus aculeatus*), pike and eel (*Anguilla anguilla*) are present at lower densities in both streams. Potential terrestrial and avian predators present in the adjacent area of the streams include mink (*Neovison vison*) and grey heron (*Ardea cinerea*). Most of the trout are anadromous, migrating to sea after two years in the stream (Dellefors and Faremo, 1988).

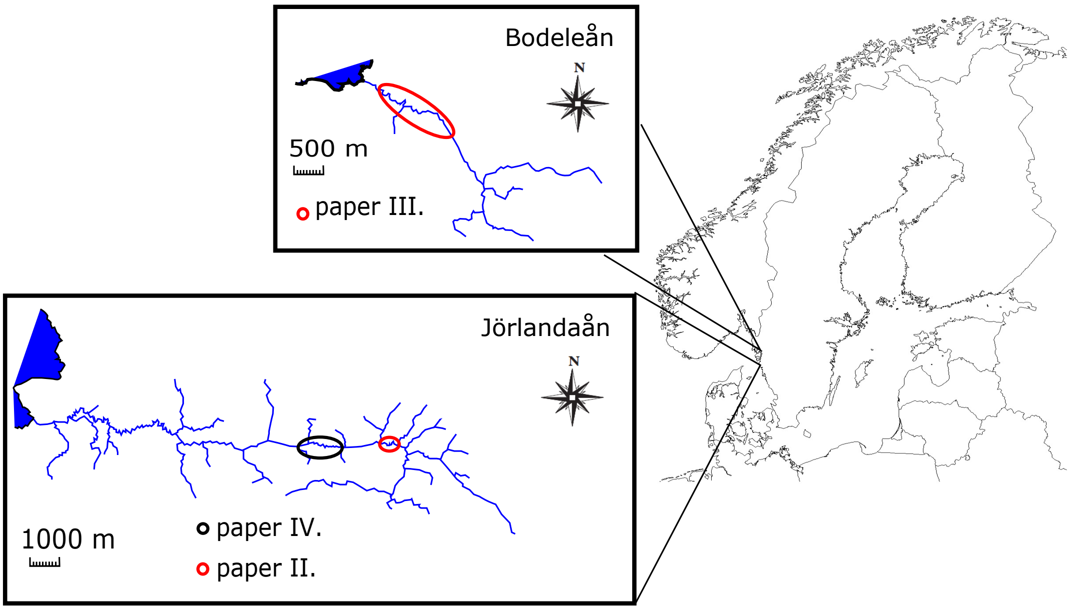

Figure 4 - Map of sampling sites west coast of Sweden used for data collection.

Figure 5 – Example of a typical landscape and vegetation at the sampling sites on west coast of Sweden

Fish sampling and manipulation

All of the experimental procedures were complied with valid legislative regulations of Czech Republic, Sweden and EU. All experiments were carried out with wild brown trout and experiments on west coast of Sweden were focused only on freshwater life stages of anadromous populations. Fish were caught by standardized electrofishing using 200-300 V of direct current (Bohlin et al., 1989). Before other manipulation, trout were anaesthetized (2-phenoxyethanol; 0.5 ml · L^{-1}), and measurements of body length and weight were taken. When tagging was to take a place it followed after this procedure. Experimental fish were tagged by 12 mm PIT-tags (Oregon RFID, Portland, OR, USA or Trovan, UK) into the body cavity or by Visible Implanted Alphanumeric and Visible Implanted Elastomer Tags (Northwest Marine Technology, WA, USA) implanted into the skin (Olsen and Vøllestad, 2001).

Dispersion of individuals across the observed streams was determined based on distance between the location of tagging and recapture or by active telemetry using portable RFID antennas (Oregon RFID, Portland, OR, USA).

Three methods were used in this thesis to assess growth and age of individuals:

1) Scale reading. In the studies where scale reading was applied (papers **I**, **V**, **VI**), back calculation of growth was used according to Martinson et al. (2000) using the Fraser-Lee equation (Lee, 1920). Age was determined using a standard notation following Pearson (1928).

2) Difference in body size was measured using two consecutive measurements. This method was used in the studies **II**, **III** and **IV**. Fish were measured either directly by scale or measuring board or by image analysis using ImageJ 1.46r software (http://imagej.nih.gov), when the size increment was assessed from a chronological sequence of photographs of a individual. The age of individuals in these studies was determined based on known length-frequency distribution of cohorts in the studied populations and scale reading in problematic cases.

Samples of scales for determination of growth and age (papers **I**, **V** and **VI**) were taken from the left body side, one row above the lateral line in the intersection of dorsal and ventral fin (DeVries and Frie, 1996). Tail fin clip of tissue was taken from 188 individuals for genetic analyses in paper **VII**. A plastic disposable 7 ml Pasteur pipet (VWR International, Radnor, PA, USA) filled with water was used to flush out the stomach content in paper **III.** The pipette was inserted through the oesophagus of the fish and the water was pressed into the stomach while keeping light pressure on the abdomen of the fish. This procedure forced back the water out through the oesophagus bringing with it the stomach contents. This flushing method follows similar principles as the syringe flushing method described by Meehan and Miller (1978).

When kept in captivity fish were housed in glass laboratory tanks. The laboratory holding tanks (paper **II** and **III**) were provided with gravel, shelters (rocks and plastic plants) and fresh water from a flow-through filtration system (flow rate 2 L min^{-1}). Photoperiod followed natural day–light cycles and temperature was kept at 11 – 13°C throughout the experiment in both holding tanks and experimental tanks. Mortality of individuals kept in the lab was generally low, there was no laboratory mortality among the fish used in the experiment described in the paper **II** and mortality in the paper **III** was 7 %.

Statistical analyses

Statistical analyses were performed using statistical software SAS (Version 9.1, SAS Institute Inc., USA) and R (Version 3.1.0, R Core Team, Austria). The value of the fixation index (F_{ST}) as an indicator of genetic divergence was calculated by software 6 Arlequin 3.11 (Excoffier et al., 2005).

Associations between the variables related to the inter-individual variation in growth, behaviour and age were tested using generalized linear models (GLM). The data were transformed for normality when necessary. To account for repeated measures, some analyses were performed using a linear mixed model with (LMM) random factors. The significance of each fixed effect in the GLM and LMM was assessed using an F-test. The degrees of freedom were calculated using the Kenward-Roger method (Kenward et al., 1997). For multiple comparisons a Tukey-Kramer adjustment was used.

The population growth rate was used in the paper **VII** instead of raw numbers of individuals, to avoid a bias in correlation toward a long-term trend in population size (Bjørnstad et al., 1999). Because of non-independence of the pairwise data, the Mantel test (10 000 permutations) was performed to verify the correlation between demographic synchrony, genetic divergence and spatial distance (Dinzin-Filho et al., 2013); significance levels were corrected using sequential Bonferroni method (Rice, 1989).

Short summary of each paper

I Závorka, L., Horký, P., Höjesjö, J. and Slavík, O. *Effect of individuals' local persistence, spatial and temporal scale on density-dependent growth: a study in brown trout*

This study focused on the mechanism of density-dependent growth in a stream dwelling landlocked population of brown trout *Salmo trutta*. The specific questions proposed were: (1) Is density dependent growth altered by ontogeny? (2) Is strength of density dependent growth affected by individual's persistence within the locality? (3) Does the effect of density dependent growth differ on the spatial and temporal scale? To answer these questions, body growth estimated by scale reading was examined in relation to population density and recapture rate of individuals across two seasons (spring and autumn) and two spatial scales (a reach and a whole stream). In concordance with previous studies (Imre et al., 2005; Vincenzi et al., 2010), the whole stream population density had a negative effect on body growth in juveniles, but not in adult individuals. However, negative density dependent growth was detected only in the reaches with low recapture rate whereas no such relationship was found in reaches with high recapture rate.

It has been shown, that exploitative and interference competition (Davies, 2012) are important for regulation of density dependent growth in salmonid fishes (Imre et al., 2005; Ward et al., 2007; Imre et al., 2010; Lindeman et al., 2014). Population density on the large-scale (several kilometres or the whole stream) can drive depletion of resources and changes the growth of individual throughout exploitative competition. On the other hand, direct competitive interferences within a local group may exclude individuals from their preferred habitat (Vehanen et al., 1999) or food resources (Nakano et al., 1999) and thereby increase their stress level and associated energetic costs (Kaspersson and Höjesjö, 2009).

Therefore it is possible that persistence within a certain locality increases familiarity of individual with habitat and with other conspecifics (Dukas, 2004), which mitigates aggressive interactions among competing individuals (Höjesjö et al., 1998). Consequently, relaxing effect of familiarity towards conspecifics and with habitat could minimize the negative influence of population density on growth of individual within the reaches with high recapture rate.

II Závorka L., Näslund J., Aldvén D., Höjesjö J. and Johnsson J. *Familiarity mitigates effects of density dependent competition: an experimental study on territorial salmonid fish*

Deleterious effect of competition for space and food in animals increases with increasing population density (Elliott, 1994; Scott, 1988). In contrast, familiarity towards conspecifics can relax intensity of interference competition (Höjesjö et al., 1998). This papers' hypothesis is that familiarity towards conspecifics and habitat mitigates effect of density-dependent growth and dispersal in territorial animals. To test this, growth of wild-captured juvenile brown trout held in tanks under a 2x2 factorial density and groups stability (i.e. familiarity) experimental design was observed for 40 days. Individuals were subsequently exposed to emergence test giving them option to leave their group and shift to a novel unoccupied environment. The results show that familiarity increases growth in high-density groups, whereas only weak effect was found in the low-density groups. Individuals within the familiar groups had also lower level of fin erosion (the consequence of aggressive interactions - Cañon-Jones et al. 2011). Growth of individuals was strongly affected by their size rank within the group, reflecting dominance status, with the largest individuals growing disproportionally faster than the rest of the group. However, the second and third fish in the size rank did not grow significantly faster and tended to suffer higher mortality than the rest of group. The largest individuals in the familiar groups left the shelter during the emergence test either as the first (6 out of 12 groups) or as the last in the sequence (5 out of 12 groups) from the whole group, while no such pattern was observed among unfamiliar individuals. In summary, this study showed that the mitigation of aggressive interactions by familiarity in the stabile groups despite the increasing population density, which has a positive effect on growth rate of all individuals within the group. However, familiarity seemed to be especially advantageous for dominant individuals, which apart of the superior growth and were less constrained by other group members in behavioural decisions they made in the emergence test (Adriaenssens and Johnsson, 2013). This might be especially important in highly fecund organisms like fish that undergo density-dependent bottlenecks during early-life (Elliott, 1994; Kaspersson and Höjesjö, 2009).

III Näslund J., Aldvén D. and Závorka L. *Eggs from anadromous adults provide marine-derived nutrients to Atlantic salmon and brown trout parr prior to the onset of winter – observations from a Swedish coastal stream*

This paper shows that the eggs from anadromous salmonids can constitute a large proportion of the ingested food for juvenile Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* during the spawning period in late autumn. Eggs were found to be consumed by the majority of the fish with fork lengths over 80 mm residing in the spawning areas. Brown trout were found to be able to consume eggs at a size of 68 mm fork length (young-of-the-year). The corresponding size for Atlantic salmon was 79 mm. The eggs of anadromous salmonids provide a rich source of nutrients, derived from marine environments, to the freshwater residing juveniles at the onset of the winter season. We highlight that eggs may constitute an important energy input for juvenile salmonids in anadromous populations in Europe, and could possibly influence overwinter survival and subsequent smoltification in the following spring. Active egg predation at the moment when the female releases her eggs could be associated with substantial risks as both females and males defend the nest against intruders duing the spawning (Fleming, 1998). A study by Tentelier et al. (2011) suggests that male brown trout exhibit short-term parental care, as they appear to stay for a short while on the nest site to keep trout cannibals away from the newly laid eggs. Therefore, egg eating is behaviour with potential consequence for trade-off between growth and mortality and might be associated with other behavioural traits within a pace-of-life syndrome of an individual (Réale et al., 2010).

IV Závorka L., Aldvén D., Näslund J., Höjesjö J. and Johnsson J. *The fluctuating cost of high activity – an extension of the Pace of Life Syndrome hypothesis*

Theory suggests that consistent inter-individual differences in activity are linked to life-history where high activity is associated with rapid growth, high dispersal tendency, and low survival (the pace-of-life syndrome hypothesis - POLS) (Réale et al., 2010). This study addressed this hypothesis by combining behavioural studies with fine-scale positional scoring in nature, estimating how individual movement strategies in brown trout associate with fitness correlates (growth and survival) in the wild. Initial dispersal in the wild was positively related to the laboratory activity. Moreover, the growth of individuals with high laboratory activity decreased with increasing home range size, whereas the growth of individuals with lower laboratory activity increased slightly with increasing home range size. Survival in the wild was not associated with laboratory activity. These results do not support the original pace-of-life syndrome hypothesis. Me and coauthors suggest along with previous studies (Dingemanse et al., 2004; Dingemanse and de Goede, 2004; Adriaenssens and Johnsson, 2009, 2013; Montiglio et al., 2014) that the POLS hypothesis in its original form is too simplistic to explain the adaptive value of consistent behavioural traits under natural conditions, as exemplified by our stream model system, a complex and unpredictable environment with fluctuating selective pressures (Höjesjö et al., 2004). As an alternative explanation, we suggest that the growth of individuals adopting a high-activity strategy is more sensitive to variation in resource abundance (indicated by home range size) than the fitness individuals adopting a more passive strategy.

V Závorka, L., Slavík, O. and Horký, P. *Scale reading estimates validation in brown trout Salmo trutta population dwelling in mountain headwaters*

The aim of this study was to validate age and growth estimates from scale reading in a landlocked population of brown trout Salmo trutta L., 1758. Some previous studies showed substantial variation in accuracy of scale reading across populations of fishes (for example Hoxmeier et al., 2001). Therefore, this validation was also important for the method of this thesis, because growth and age estimations in papers **I** and **VI** were based on scale reading from the same population. The estimates from scale reading were validated by comparison of results obtained from a sample of 53 repeatedly caught individuals. The estimated age of the captured fish ranged from 1+ to 8+. The age-corrected absolute percentage error was 10.71%, primarily because the ages of the oldest individuals according to scale reading were underestimated, and the ages of juvenile individuals were slightly overestimated. The back-calculated length was slightly underestimated (the mean error was −4.60 mm), but it was not significantly different from the real measured length. This study suggests that in cold oligotrophic waters, scale reading is a sufficiently accurate method for age and growth estimation for juvenile individuals until maturity, which is in the observed streams represented by age classes 0+ and 1+ (Baruš and Oliva, 1995).

VI Závorka, L., Slavík, O. and Horký, P. *Individual growth and population distribution of brown trout in pristine headwaters*

The aim of this study was to investigate spatial distribution and the individual growth patterns of brown trout across three age groups in the headwaters of a small mountain catchment in Czech Republic. Data from this sample were based on spring and autumn sampling during the period 2005–2011 on twenty sites in the headwaters of the Otava River. The sampling sites were grouped into fourteen so-called synchronized population units within the boundaries of three populations, based on migratory connection. The individual growth of juveniles (age-0, age-1) varied significantly between all three spatial units (sampling sites, synchronized population units and populations), while the growth of adults (age-2 and older) did not. The differences in individual growth associated with migratory isolation among the observed population units indicates that a metapopulation structure can occur in the observed watershed (Koizumi 2011). Therefore, further examination of the population structure based on demo genetic analyses could have significantly improved the applicability of these results for conservation management of the studied population.

VII Závorka L., Horký P., Kohout J., Kalous L. and Slavík O. *Demogenetic structure of brown trout Salmo trutta Linnaeus, 1758 populations in mountain headwaters: implications for conservation management*

This study was focused on the demographic and genetic structure of a wild brown trout population within a small headwaters system of the Otava River in Czech Republic. Specific aims of this study were to: (i) examine the demographic and genetic pattern of a brown trout population with respect to the connectivity of the headwaters system; and (ii) examine the isolation among the population units, which differ by growth rate of juvenile individuals; (iii) determine basic management units for the studied landlocked populations of brown trout. A demographic analysis was based on the same dataset of 7 years long observation used in the paper **VI**. A genetic analysis was based on fin clip samples of 188 individuals collected between the years 2005 and 2009. Synchronized population units from the paper **VI,** which differ by growth rate of juvenile individuals were used here as a basic sampling unit (see Fig. 3). The value of genetic divergence (F_{ST}) among sampling units ranged from -0.03 to 0.16. Demographic synchrony was low or moderate, and the average correlation coefficient of population growth between sampling units (r) ranged from 0.28 to 0.66. No isolation by distance was observed, but genetic divergence was negatively correlated with demographic synchrony among sampling units. Variance in the population growth rate (i.e. local extinction probability – see Green 2003) increased with distance from the mainstream and from other sampling units. In contradiction with the usual model of stream-dwelling salmonid fishes (Klemetsen et al., 2003; Quinn, 2005), the upstream sections of headwaters held only ephemeral subpopulations, whereas the mainstream played a role in the source area of the metapopulation. These findings stress the importance of the mainstream in management conservation for brown trout in low productive mountain headwaters.

Conclusions

This thesis explored how environmental factors affect body growth and survival of brown trout. It was particularly focused on the effect of population density on these two life-history traits and associated underlying processes. The major results of this thesis could be summarized in the following points:

(1) Group stability increasing familiarity towards conspecifics and with the environment relaxes the intensity of agonistic interactions among juvenile individuals of brown trout. Therefore, group stability can mitigate the negative effect of increased population density on growth in the natural environment throughout the moderation of interference competition.

(2) There are consistent inter-individual differences in activity in a population of brown trout corresponding to individuals' animal personality. The personality traits of an individual (represented here by activity) associate with growth, survival and dispersal in field. Specifically, more active individuals disperse further and adopt a high gain/high cost strategy and are therefore able to yield a high net energy income and growth rate when conditions are optimal (e.g., high food availability). However, they suffer by low net energy income and growth rate under suboptimal conditions (low food availability) due to high activity–related maintenance costs. In contrast, less active individuals are less dispersive and adopt a low gain/low cost strategy, allowing them to maintain a more constant growth rate under a wider range of environmental conditions.

(3) Wild population of brown trout in a small mountain watershed, represented here by the headwaters of the Otava River in Šumava NP (Czech Republic), consisted of demographically and genetically distinct subpopulations, which displayed differences in survival and growth rate of individuals. The upstream sections of the studied headwaters held only ephemeral subpopulations, whereas the mainstream played a role in the source area of the metapopulation.

This thesis reports several examples, which stress the importance of behavioural adaptations and group stability in density dependent processes. The importance of local adaptations for growth and survival was in this thesis demonstrated by an example of wild populations of brown trout from the Swedish west coast which was almost completely unaffected by artificial stocking. I showed with my co-authors that populations of brown trout consist of a variety of different animal personalities (behavioural types), which are consistent across time and context and associate with the ability of individuals to utilize resources. The differentiation of strategies within a population can be a consequence of parental bed hedging strategy (Hammer et al. 2002), fluctuating selection (Dingemanse et al., 2004) or density dependent selection (Maynard-Smith 1982). Thus, despite the general validity of negative relationship between population density and growth and survival, local behavioural adaptations of individuals can cause differences in the strength of this process among populations of brown trout.

Differences in life-history traits among genetically and demographically isolated subpopulations in the small watershed of the Otava River headwaters indicates the high value of local adaptations for the viability of the wild populations of brown trout. A glance of recovering local adaptations in the population might be of high importance in central Europe, where the original genotypic variability of brown trout has been lost (Kohout, 2012). This process might be further reinforced by conservational management on brown trout population dwelling in lower stretches of Otava River, which seems to be the source subpopulation connected with the less stabile subpopulations located higher upstream within the National Park (see Koizumi, 2011).

The extensive artificial stocking, which has been applied in lakes and rivers of the eastern and central Europe (Kohout, 2012), can facilitate propagation of maladaptive phenotypes within a population. This can cause erosion of local adaptations of individuals on variation of population density. Therefore, substantial reduction of stocking of hatchery raised fish together with sensitive management protecting spawning sites and large parental individuals could have a positive effect on populations of brown trout (Waples, 1991; Ryman et al., 1995).

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Attached Publications

PAPER I

Závorka L., Horký P., Höjesjö J. and Slavík O. (2015) Effect of individuals' local persistence, spatial and temporal scale on density-dependent growth: a study in brown trout.

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Effect of individuals' local persistence, and spatial and temporal scale, on density-dependent growth: a study in brown trout *Salmo trutta*

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This study focuses on the mechanism of density-dependent growth in a stream-dwelling landlocked population of brown trout *Salmo trutta*. Specifically, body growth estimated by scale reading was examined in relation to population density and recapture rate (approximation of persistence of individuals within a location associated with dispersal and mortality) across 7 years and two spatial scales – a reach and a whole stream. In concordance with previous studies, the whole-stream population density had a crucial effect on body growth in juveniles, but not in adult individuals. Furthermore, growth was negatively associated with the whole-stream population density in reaches with a low recapture rate, whereas no such relationship could be detected in reaches with a high recapture rate. Since persistence within a certain locality increases the familiarity of individuals with the habitat and with other conspecifics, we suggest that the negative effect of population density on growth might be relaxed in groups with a high recapture rate by positive influence of the familiarity both within the habitat but also towards other conspecifics, which decreases competition pressure.

KEY WORDS: behaviour, familiarity, intraspecific competition, fish, streams.

INTRODUCTION

A negative relationship between population density and the condition of individuals, induced by a decrease in available food and space and an increase of stressful interactions, is widespread among animal species (Scott 1988; Harrison et al. 2011). Body size-at-age as a fundamental condition factor determined by population density

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could affect important life traits such as fertility, maturity, survival and migratory behaviour, particularly in vertebrates with undetermined body growth, such as fishes, amphibians and reptiles (Jonsson et al. 1991; Scott 1994; Fordham et al. 2007). The importance of density-dependent growth is likely to vary both spatially and temporally and may affect the survival and dispersal of individuals, which as sympatric processes can reduce the intensity of density-dependent growth (Nakano 1995; Einum et al. 2006; Lobón-Cerviá 2007). High emigration and low survival in a dense population can induce and alter the timing of ontogenetic development such as metamorphosis, which decreases competition pressure and thus improves the condition for the remaining individuals in the particular life stage. For instance, tadpoles of spadefoot toad *Scaphiopus holbrooki* exposed to high population density displayed lower survival rates, metamorphosed at the minimum size and left the pool earlier than tadpoles exposed to lower population density. As a consequence, the remaining tadpoles experienced more favourable growth conditions (Semlitsch & Caldwell 1982).

In order to detect density-dependent processes, appropriately scaled studies need to be conducted (Ray & Hastings 1996). Studies addressing the density-dependent growth in mobile organisms should focus on a spatial scale large enough to capture the density of conspecifics experienced, but small enough to prevent averaging the density of the independent local populations, whose dynamics may be incommensurable (Hassell 1987; Jenkins et al. 1999). The mobility and surviving capacity of vertebrates with undetermined body growth typically increase during ontogeny, as a function of body size (Einum & Nislow 2005; Einum et al. 2006; Parra et al. 2011), but can also vary among populations (Bohlin et al. 2001; Massot et al. 2002). Thus, the temporal and spatial range of density-dependent growth may differ significantly among populations within species (Ward et al. 2007).

Salmonids, as territorial species (Kalleberg 1958), with high fecundity, complex life history (Klemetsen et al. 2003) and undetermined body growth, are a good object for examining mechanisms of density-dependent growth. Field and laboratory studies have shown that the effect of population density in salmonids is mirrored in the survival and dispersal of individuals across life stages (Elliott 1984; Jonsson et al. 1998; Lobón-Cerviá 2012), but also in the body growth of juveniles (Elliott 1988; Bohlin et al. 2002; Brännäs et al. 2004; Kaspersson & Höjesjö 2009). However, the spatial scale at which density-dependent growth of juvenile salmonids has been reported varies significantly among studies, from tens of metres (Crisp 1993) up to thousands of metres or the whole stream (Imre et al. 2005; Vincenzi et al. 2010). Juvenile salmonids are generally believed to be affected by density-dependent growth and dispersal at low densities, but density-dependent survival at high densities (Jenkins et al. 1999; Imre et al. 2005; Lobón-Cerviá 2007). However, this pattern is affected by dispersal ability and survival capacity in the early ontogenetic phase (Einum et al. 2006; Parra et al. 2011). Populations with high survival and low mobility should be more susceptible to density-dependent growth than populations with low survival and high mobility are. Although dispersal and mortality are ecologically different processes, they both cause individuals to disappear from the population, and it might be challenging or even impossible in field studies to distinguish between them. However, both dispersal and mortality are reflected in recapture rate (Kaspersson & Höjesjö 2009), and may provide insight into individuals' persistence within localities and groups of conspecifics.

Here, we provide the results of a long-term field study concerning density-dependent growth at two spatial scales (reach and stream) during 7 consecutive years, using brown trout *Salmo trutta* as a model organism. The specific questions proposed in the study were: (1) Is density-dependent growth altered by ontogeny? (2) Is the strength of density-dependent growth affected by individuals' persistence within the locality? (3) Does the effect of density-dependent growth differ on the spatial (reach, stream) and/or temporal (spring, autumn) scale?

METHODS

Over the 7 years, 5195 brown trout were caught in the headwaters of Otava River (49°1′N, 13°29′E), Elbe catchment area, Czech Republic, which is found within a protected area of the Bohemian Forest National Park. A detailed description of the study area, fish population and sampling surveys has been given elsewhere (Závorka et al. 2013); therefore, only aspects relevant to this study are reported here. The observed headwaters are drained by two main streams, Vydra River and Křemelná River, both approximately 30 km long. No obstacles prevent trout migration in the system; the only exception is a tributary of Vydra River, Švelský Stream, which is isolated by a natural waterfall from the rest of the system, and was therefore considered an independent stream, in all analyses. Brown trout was the only fish species detected during this study in the Vydra River and Šveský Stream. In Křemelná River, European bullhead *Cottus gobio* was occasionally detected. The examined populations of brown trout in this study are all stream residents; there are no trout with a migratory life cycle associated with larger water bodies (lakes, larger rivers). Otava River below the confluence with Křemelná and Vydra has an average discharge of 8 m^3 sec^{-1} and it still has the character of a mountain trout stream. The majority of trout fry in the watershed emerge from the gravel during late May and early June.

Fish were sampled twice a year (May and October) during the period from autumn 2005 to autumn 2011 in 20 sampling sites, and caught with a backpack electroshocker (EFKO, Germany). With regard to welfare handling of observed animals, a single-pass electrofishing method was used, which is considered sufficient for the determination of brown trout abundance in mountain headwater streams (Kruse et al. 1998; Cattanéo et al. 2002). Sampling effort, sampling site area and position were kept intact, and temperature (mean ± SD: 8.1 ± 2.4 °C) and stream conductivity (mean ± SD: 22.0 ± 5.2 μS cm^{-1}) before each sampling were also similar among the sampling sites throughout the study. Following the recommendations for an electrofishing sampling protocol (Bohlin et al. 1989), catch efficiency among samplings was therefore assumed to be similar. Every specimen was anesthetised (2-phenoxiethanol, 0.5 mL L^{-1}), measured (standard length to the nearest mm), weighed (to the nearest g) and individually tagged in the jaw using visible implant alphanumeric tags (Northwest Marine Technology, WA, USA). Specimens that were of insufficient size for individual tagging (standard length smaller than 90 mm) were marked using visible implant elastomer (VIE) tags (Northwest Marine Technology, WA, USA). The detection of tagged fish was noted in the recapture protocol. After this procedure, all fish were released to the same place from which they were caught. Stream slope, stream bed substratum and average water discharge were measured once, at the beginning of the study (Table 1).

Data analyses

To ensure good comparability of growth data, independently on any variation of the recapture rate among sampling sites, the individual growth and age of fish were only estimated via scale readings performed along the anterior–posterior axis of the scales (Martinson et al. 2000). This analysis was conducted on a subsample of 709 randomly selected individuals, from which 5–10 scales in a row above the lateral line were removed (Devries & Frie 1996). Growth was quantified as a between-year increment of length-at-age back-calculated from the scale analysis. Due to a well-known high level of estimation errors that occur in scale reading of salmonids older than 3 years (Alvord 1954), these individuals were grouped in a single category for all analyses. Therefore, we distinguished four age groups represented by juveniles in age groups 0+ and 1+, by adults in age groups 2+, and by a pooled group of adult individuals equal to

Table 1.

Values of tested abiotic factors and lengths of sampling sites, and their distribution within observed reaches and streams.

Stream	Reach	Sampling site	Sampling site length (m)	Slope (‰)	Dominant substrate (%)	Discharge (m^3)
Křemelná	1	K1	130	0.9	Gravel (64)	–
Křemelná	2	K2	328	1.24	Gravel (45)	0.62
Křemelná	2	K3	256	1.6	Gravel (48)	–
Švelský	3	S	274	35	Boulders (51)	0.14
Vydra	4	H2	171	9.56	Pebbles (38)	0.44
Vydra	5	V	224	2.45	Pebbles (53)	2.1
Vydra	6	H1	201	2.34	Gravel (57)	0.18
Vydra	7	F	183	3.56	Pebbles (48)	0.08
Vydra	8	M1	172	2.78	Pebbles (57)	0.34
Vydra	9	M2	191	2.43	Boulders (42)	0.59
Vydra	9	R3	208	1.6	Pebbles (52)	1.13
Vydra	10	B	185	6.13	Pebbles (59)	0.09
Vydra	10	L2	134	0.89	Gravel (51)	0.21
Vydra	11	L1	100	1.56	Gravel (75)	0.21
Vydra	12	R1	216	2.27	Gravel (83)	0.09
Vydra	12	J2	176	1.87	Pebbles (52)	–
Vydra	13	R	195	0.68	Gravel (83)	0.11
Vydra	14	J1	170	1.72	Pebbles (64)	0.22
Vydra	14	T	290	3.78	Pebbles (57)	0.32
Vydra	15	R2	193	1.87	Pebbles (52)	1.14

or older than age 3+. According to Závorka et al. (2014), precision of scale reading in the focal population was found to be sufficiently precise in juvenile individuals, but the reliability of growth and age estimates in older individuals might be limited.

For all analyses, the total population density (number of caught individuals divided by area of the sampling site) was used. This measure was used because it is comparable across the temporal and spatial axis of the study; although the catch effort per sampling within the sampling site was the same across the years, the sampled area differed between sampling sites. Sampling sites were spread out equally within the stream length. Abundance in the observed streams was too low to produce reliable estimates (in some, years there were one or no individuals caught in some sampling sites); therefore, to address our questions, sampling sites were pooled into larger units – streams and reaches. The pooling of the sampling sites resulted in 15 reaches (the average area of pooled sampling sites was 546 m^2, SD = 553, range from 50 m^2 to 1680 m^2), which were delineated based on mutual migratory connections and three streams (Vydra, Křemelná Rivers and isolated tributary of Vydra River/Švelský Stream; average area of pooled sampling sites: 6801 m^2, 1771 m^2 and 272 m^2 for Vydra River, Křemelná River and Švelský Stream respectively). The migratory connection among the streams was limited by distance, and by the migration barrier in the outlet of Švelský Stream. The migratory connection among the sampling sites was estimated based on the movements of tagged individuals; specifically, when an individual tagged at one sampling site was recaptured at other sampling site, these sampling sites were considered as migratorily connected and they were pooled into one reach. No migration among the streams was observed during the study. Trout numbers and the area used for calculating the density at a spatial scale were obtained by pooling all the trout and the area, respectively, of the sampling sites integrated into that spatial scale (Imre et al. 2005). Similarly, the values of abiotic factors at the reach and the stream level were calculated as an average value of the sampling sites integrated into that spatial scale. To test seasonality in density-dependent growth (Ward et al. 2007; Helland et al. 2011), growth–density relationships were modelled from the initial (spring) and final (autumn) density in a season. The recapture rate was calculated as the percentage of recaptured individuals relative to the total number of tagged individuals within a reach during the whole study. The recapture rate was used as an indicator of individuals' local persistence within the observed reaches. To exclude the alternative that variation in the recapture rate was caused by differences in the effectiveness of electrofishing, a correlation test between the recapture rate and all available environmental variables which could potentially affect the effectivity of electrofishing was performed. We found no significant relationship among these variables and recapture rate (Spearman's rank correlation for water temperature before the sampling $\rho = 0.36$, $P = 0.18$; conductivity before the sampling $\rho = 0.33$, $P = 0.24$; stream width $\rho = -0.01$, $P = 0.99$; sampling area $\rho = 0.06$, $P = 0.84$; stream slope $\rho = 0.30$, $P = 0.27$; and Kruskal–Wallis test for the stream bed substratum $\chi^2 = 1.96$, $P = 0.375$). Therefore, we assumed that recapture rate is a good proxy of individuals' persistence within the reach.

Although comparable within our study, the recapture rate derived by one-pass electrofishing could underestimate the persistence of individuals, because catch efficiency (Bohlin et al. 1989, approximately 50%) and retention of VIA tags (Rikardsen et al. 2002, approximately 78%) are limiting factors. Therefore, the recapture rates used in our analyses may not be directly comparable with recapture rates in other studies.

Statistical analyses

The statistical analyses were performed using the SAS software package (SAS Institute Inc., version 9.2, NC, USA). The data were transformed to meet normality requirements when needed.

Due to a low recapture rate, PROC RANK analysis was used to partition data into two groups on the basis of values of recapture rate (see results for further information). The growth data were analysed using a linear mixed models (LMM) with random factors (PROC MIXED). The random factors were used to account for repeated measures across the duration of the experiment. The significance of each exploratory variable (i.e. fixed effect, including their interactions) in the particular model was assessed using an F-test. The degrees of freedom were calculated using the Kenward–Roger method (Kenward & Roger 1997).

RESULTS

The strength of density-dependent growth differs depending on the spatial scale between reach and stream and also on the temporal scale between spring and autumn. In detail, density-dependent growth relationships were only shown to be significant when using the initial (i.e. spring) population density (Fig. 1) and the whole-stream spatial scale. At the scale of reaches and during the autumn season, no effect of population density on brown trout growth could be detected (Table 2). There was no significant correlation between the spring and autumn trout densities at the level of reaches (Spearman's rank correlation $\rho = 0.198$; $P = 0.061$) and streams (Spearman's rank correlation $\rho = 0.046$; $P = 0.854$). Further, no effect of stream slope, stream bed

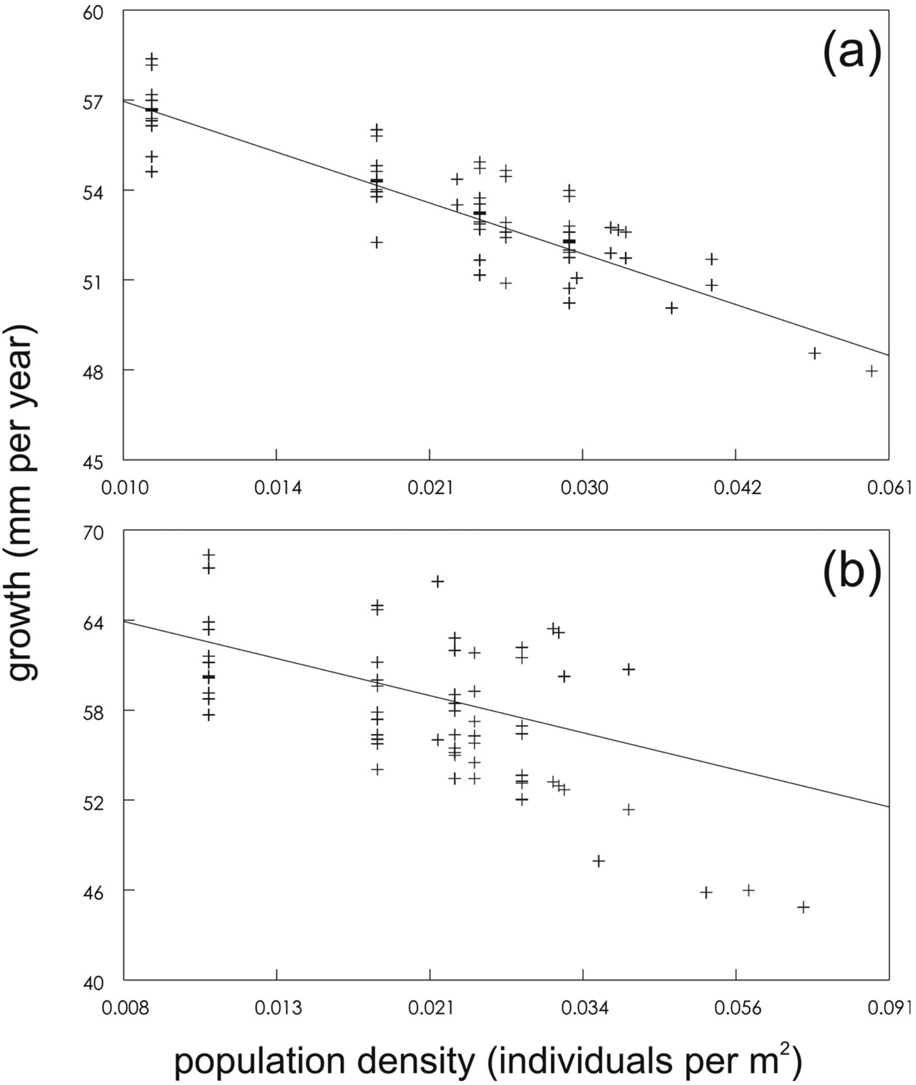

Fig. 1. — Plot of the relationship between the growth of (a) 0+ trout (n = 514; R^2 = 0.84; P < 0.01) and (b) 1+ trout (n = 395; R^2 = 0.24; P < 0.01) and the stream population density in spring. Data are pooled for all 15 reaches, both groups of reaches with high and with low recapture rates are included.

substratum or average water discharge on growth of individuals was detected, either at the reach or the stream spatial scale.

Despite the fact that the growth of individuals was affected by the whole-stream population density, there was an influence of individuals' persistence within the reach on the strength of this density-dependent relationship. The average persistence of individuals represented by the recapture rate at the reaches during this study was 6.79% (SD = 3.99), ranging from 0.00 to 15.52%. PROC RANK revealed that the

Table 2.

Type-3 tests of fixed effects for a relationship between growth and population density. The origin of data for particular models is indicated (spatial scale, age group and season). Significant tests (*P* value > 0.05) are given in bold face.

Population density spatial scale	Age group	Season	Persistence of individuals (recapture rate)	Num df	Den df	F value	*P* value
Reach	0+	Spring	Pooled	1	371	1.10	0.2957
Stream	**0+**	**Spring**	**Pooled**	**1**	**137**	**7.14**	**0.0085**
Reach	0+	Autumn	Pooled	1	460	0.00	0.9500
Stream	0+	Autumn	Pooled	1	26.3	1.01	0.3240
Reach	1+	Spring	Pooled	1	393	0.09	0.7687
Stream	**1+**	**Spring**	**Pooled**	**1**	**37.4**	**9.96**	**0.0031**
Reach	1+	Autumn	Pooled	1	457	0.87	0.3528
Stream	1+	Autumn	Pooled	1	52.6	0.02	0.8772
Reach	2+	Spring	Pooled	1	129	0.00	0.9775
Stream	2+	Spring	Pooled	1	129	0.25	0.6180
Reach	2+	Autumn	Pooled	1	143	0.05	0.8236
Stream	2+	Autumn	Pooled	1	143	0.17	0.6825
Reach	≥3+	Spring	Pooled	1	12	0.49	0.4962
Stream	≥3+	Spring	Pooled	1	12	1.02	0.3317
Reach	≥3+	Autumn	Pooled	1	14	0.42	0.5295
Stream	≥3+	Autumn	Pooled	1	14	0.01	0.9338
Stream	0+	Spring	High	1	294	2.42	0.1205
Stream	**0+**	**Spring**	**Low**	**1**	**148**	**5.91**	**0.0162**
Stream	1+	Spring	High	1	1.98	1.19	0.3904
Stream	**1+**	**Spring**	**Low**	**1**	**124**	**7.83**	**0.0059**

boundary between high and low levels of recapture rate was 7.00% of recaptured individuals, and the reaches were split into two groups accordingly. The group of reaches with a high persistence of individuals (recapture rate > 7%) contained seven reaches, and the group with a low persistence of individuals (recapture rate < 7%) contained eight reaches. The growth of individuals in the reaches with a low persistence was affected by the stream population density (Fig. 2), whereas the growth of individuals in the reaches with high persistence was unaffected by the whole-stream population density.

Density-dependent growth was also altered by ontogeny. Specifically, negative density-dependent growth was confirmed for the juvenile age groups (age 0+ and 1+; Figs 1–2; Table 2) but no effect was found for older age groups (age 2+ and > 3+; Table 2).

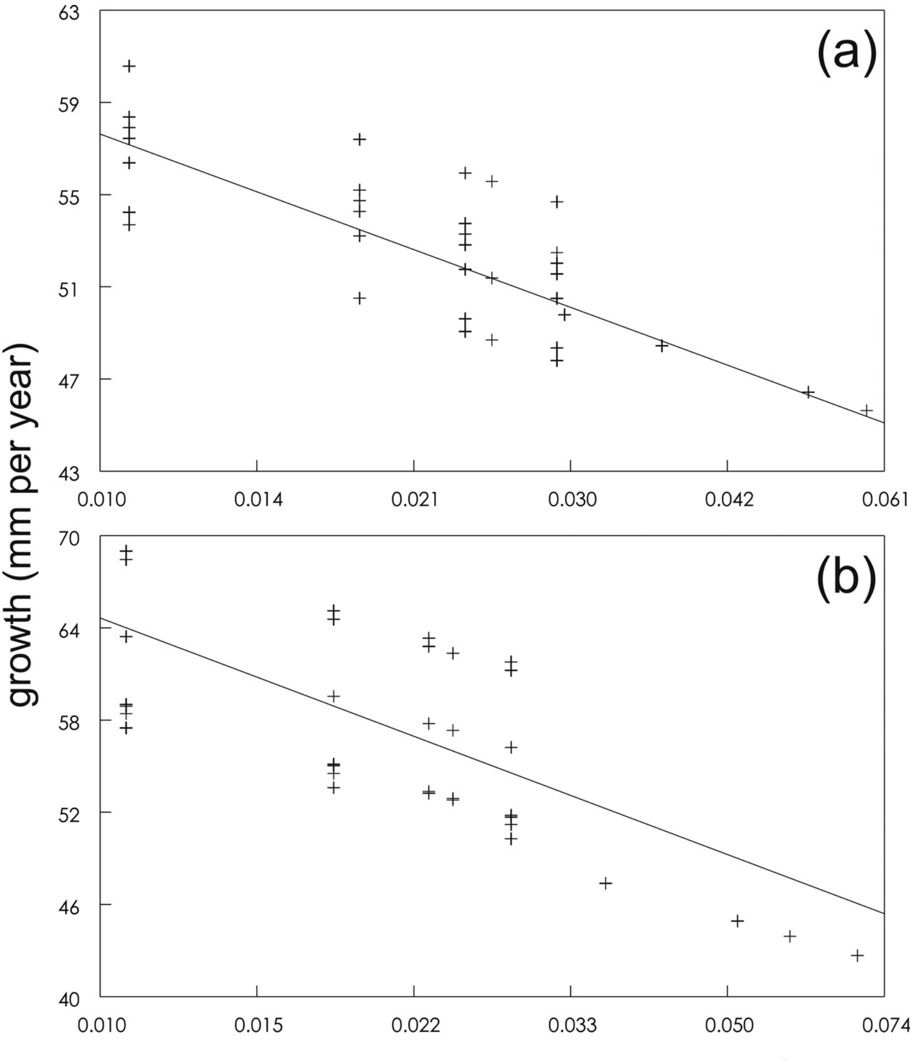

Fig. 2. — Plot of the relationship between the growth of (a) 0+ trout (n = 220; R^2 = 0.68; $P < 0.05$) and (b) 1+ trout (n = 158; R^2 = 0.50; $P < 0.01$) in the reaches with a low recapture rate (low persistence of individuals) and the stream population density in spring.

DISCUSSION

Our results showed that growth of juvenile (age 0+ and 1+) but not adult (age 2+ and > 3+) brown trout decreased inversely to the whole-stream spring population density in reaches with a lower persistence of individuals, while the negative effect of density on growth was relaxed in stream reaches with a high persistence of individuals. This finding does not fully correspond with the hypothesis assumed by some previous studies testing density-dependent growth in stream salmonids (e.g. Elliott 1988; Jenkins et al. 1999; Einum et al. 2006). The studies referred to above suggested that density-dependent growth is more intensive in less mobile groups with high survival of individuals due to strong competition for space and food and with a relatively low chance for individuals to achieve ideal free distribution (sensu Fretwell & Lucas 1969).

Competition is generally divided into exploitative and interference interactions (Krebs & Davies 1987), but the importance of these interactions in the regulation of density-dependent growth in salmonids is ambiguous (Imre et al. 2005, 2010; Ward et al. 2007; Lindeman et al. 2015). Population density on the large scale (several kilometres or the whole stream) can causes depletion of resources and changes the growth of individuals throughout exploitative competition. This is likely the case here, and in similar studies which have reported a correlation between growth rate and large-scale (i.e. whole-stream) population density (Imre et al. 2005; Vincenzi et al. 2010). On the other hand, a substantial effect of interference competition on growth cannot be ruled out even in these studies; direct competitive interferences may exclude individuals from preferred habitat (Vehanen et al. 1999) or food resources (Nakano et al. 1999) and thereby increase their stress level and associated energetic costs (Kaspersson et al. 2010). In agreement with this, Kaspersson et al. (2012) showed that the growth rate of newly emerged brown trout (0+) increased if older conspecifics were removed. We suggest that the discrepancy detected among reaches in the strength of density-dependent growth can be caused by a positive influence of familiarity both within the habitat and also towards other conspecifics, relaxing interference competition over thinned resources in the reaches with a higher recapture rate (i.e. individuals' persistence).

Individuals in reaches with a high recapture rate likely persist within the location for a longer time (i.e. the rate of outmigration and mortality is lower within these reaches; see Kaspersson & Höjesjö 2009), and could therefore establish familiarity towards the habitat and conspecifics due to the relatively higher stability of these groups (Griffiths & Magurran 1997a; Dukas 2004). This assumption has been supported by the results of a laboratory study testing the growth rate of 0+ brown trout kept in groups with different density and mutual familiarity (Závorka et al. unpubl. data). A familiar group of brown trout has been shown to establish a more stable social rank with lower aggression towards conspecifics, higher food intake and a better utilisation of food compared to unfamiliar groups (Höjesjö et al. 1998; Griffiths et al. 2004). In addition, to avoid costly interaction, subordinate individuals may employ alternative feeding tactics (Höjesjö et al. 2005, sneaky feeding) and signal their social status towards the dominant, superior individuals by a paler body colouration (Keenleyside & Yamamoto 1962; O'Connor et al. 2000). Familiarity within the habitat also enables a more efficient utilisation of resources (Johnsson et al. 1999; Cutts et al. 2002). Since familiar individuals tend to keep a closer distance to their nearest neighbours and thus naturally occur in higher densities, the negative effect of density-dependent growth in a familiar group may be further relaxed even if the density per se may be higher compared with unfamiliar groups (Höjesjö et al. 1998; Slavík et al. 2012).

The density-dependent relationship described in this study was significant only in relation to population densities in spring, but not in autumn. A probable explanation is that the spring represents a peak in productivity and growth rate of juveniles, with a more pronounced physiological response of trout (Johnsson & Bohlin 2005). It should be pointed out that our conclusions refer to the harsh environmental conditions that exist in these specific mountain headwaters, with a limited carrying capacity and a generally low population density. In more productive rivers with an abundant population, the chance to establish familiarity towards conspecifics may be limited due to a high number of inter-individual interactions (Griffiths & Magurran 1997b). In agreement, Bohlin et al. (2002) found growth to be negatively density dependent when adding the biomass of trout to a natural highly productive stream, but also found that all trout remained remarkably stationary with 85% of the recaptures being made within 20 m of release points. Hence, the effect of group stability and familiarity of individuals within the habitat and towards conspecifics on density-dependent processes may differ among streams due to regional differences in productivity, and needs to be further investigated.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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PAPER II

Závorka L., Näslund J., Aldvén D., Höjesjö J. and Johnsson J. Familiarity mitigates effects of density dependent competition: an experimental study on territorial salmonid fish.

Title

Familiarity mitigates effects of density dependent competition: an experimental study on territorial salmonid fish

Running title

Interference of individuals within a social group

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Abstract

Deleterious effect of competition for space and food in animals increases with increasing population density. In contrast, familiarity towards conspecifics can relax intensity of interference competition. Here we hypothesized that familiarity towards conspecifics mitigates effect of density-dependent growth and dispersal in territorial animals. To test this growth of wild-captured juvenile brown trout held in tanks under a 2x2 factorial density and familiarity experimental design was observed for 40 days. Individuals were subsequently exposed to emergence test giving them option to leave the group and shift to a novel unoccupied environment. The results show that familiarity increases growth in high-density groups with elevated competition, whereas only weak effect was found in the low-density groups. Growth of individuals was also strongly affected by their size rank within the group, reflecting dominance status, with the largest individuals growing disproportionally faster than the rest of the group. However, the second and third fish in the size rank did not grow significantly faster and tended to suffer higher mortality than the rest of group. The largest individuals in the familiar groups left the shelter during emergence test either as the first (6 out of 12 groups) or as the last (5 out of 12 groups) from the whole group, while no such pattern was observed among unfamiliar individuals. Our results suggest that familiarity can increase growth rate of individuals exposed to high population density. This might be especially important in highly fecund organisms like fish which undergo density-dependent bottlenecks during early-life.

Keywords: social structure, competition, personality, trout

Introduction

Increasing population density generally decreases the available resources per capita which may reduce growth and/or survival (Ostfeld & Canham 1995; Keddy 2001; Gurevitch et al. 1992; Brook et al. 2006; Harrison et al. 2011). Individuals can potentially respond to such density-dependent competition by dispersing to exploit resources in other habitats (e.g. the ideal-free distribution, Fretwell &Lucas 1970) which in turn can relax competition among remaining resident individuals (Matthysen 2005). In reality, however, dispersal often carries energetic costs (Roff 1977) as well as increased predation risk (Lima and Dill 1989). In addition, due to perceptual constraints individuals rarely have complete information about the availability and quality of alternative habitats (Abrahams 1986). Thus, dispersal is often limited resulting in competition for diminishing local resources (Einum et al. 2006).

Non-dispersing individuals may establish groups in which familiarity increases over time which potentially can reduce costs of competition (Elliot 1990; Griffiths & Magurran 1997; Kurvers et al. 2013). Indeed, repeated interactions in familiar groups has been found to reduce aggression in resource contests (Barnard & Burk 1979). Studies on several taxa, including fish (Griffiths et al. 2004) and birds (Senar et al. 1990), suggest that familiarity can facilitate the formation of stable hierarchical groups by decreasing the frequency of agonistic interactions, as the tolerance among group members increases with time (*i.e.* the shepherd hypothesis: Rohwer & Ewald 1981). Individuals can estimate the fighting ability of future opponents by watching their contests with other individuals, additional information that may save energy and reduce injuries in hierarchical groups by settling disputes faster and with less escalated aggressive interactions (Coultier et al. 1996; Johnsson & Åkerman 1998). This is beneficial as more time can be allocated to find and consume food (Seppä et al. 2001). In addition, attention can be reallocated from intraspecific interactions to vigilance towards predators (Dukas 2002), to lower the risk of predation (Griffiths et al. 2004).

Together, the discussion above suggests that familiarity among individuals could potentially mitigate negative density-dependent effects on growth and survival. However, the value of diminishing resources also increases with population density (Keddy 2001) which may counteract the familiarity effects. For example, guppies (*Poecilia reticulata*) increase their aggression toward familiar conspecifics with increasing value of contested resources (Granroth-Wilding et al. 2013). Furthermore, dominant individuals in stable groups usually have prior access to food resources, receive less aggression, and suffer lower predation risk than subordinates (de Laet 1985; Alanärä et al. 2001). Taken together this suggest that the relative advantage of familiarity for lower ranked individuals should decrease as population density increases (*i.e.* when resource limitation increases, Kokko et al. 2006).

Juvenile stream-living brown trout (*Salmo trutta*) is an excellent model species for studying interacting effects of population density and familiarity. Like many other fish species (Nikolsky 1963), brown trout are highly fecund and brood cohorts generally outnumber the carrying capacity of their environment which in turn leads to density dependent bottlenecks during the juvenile stage (Elliott 1994). Depending on population density, juvenile brown trout actively defend territories in streams and form social hierarchies with conspecifics (Jenkins 1969) where familiarity develops over time (Höjesjö et al. 1998: Griffiths et al. 2004).

Here we provide the first empirical test of the hypothesis that familiarity mitigates the negative effects of increasing population density. From this we predicted that: 1) familiarity relaxes negative density-dependent effects on growth and survival, 2) thereby reducing the dispersal of individuals to novel and potentially risky environments. We also predicted that 3) the benefits of relaxed density-dependent effects in familiar groups should be higher for high-ranked individuals due to establishment of a stronger social hierarchy which allows them to keep larger energy reserves and prior access to resources.

Materials and methods

Fish sampling and acclimation period

Fish were caught in August 2013 by electrofishing in a stream stretch between two natural waterfalls in Jörlandaån, a small coastal stream in western Sweden (N 57°58’, E 11°56’). Samples of 30 young of the year brown trout were caught from each of six 50 m-long sampling sections divided by buffering zones of the same length (180 individuals in total) and transported to a lab.

After the collection, fish were acclimatized for 24 h in six tanks (60 L, 30x32x65 cm) and sorted according to the stream section in which they were caught. The acclimation tanks were provided with gravel, shelters (rocks and plastic plants) and fresh water from a flow-through filtration system (flow rate 2 L min^{-1}). Photoperiod followed natural day–light cycles and temperature was kept at 11 – 13°C throughout the experiment in both holding tanks and experimental tanks. After the acclimation period 144 individuals (size ± SD 55.59±5.06 mm) were divided into 24 groups of six individuals. The rest of the fish were kept in the acclimation tank during the whole experiment as a reserve (Fig. 1). All experimental fish were released back to the stream after the experiment.

Experimental chambers

Fish were housed in 24 experimental chambers made from 12 glass aquaria. Each aquarium was divided into one small (20 L, surface area 663 cm^2) and one large (40 L, surface area 1386 cm^2) experimental chamber by a perforated opaque plastic divider that allowed the water to circulate between compartments, but prevented visual contact. Water outlet of aquaria and an aeration stone were placed in one experimental chamber and inlet in another, the positions of water inlet and water outlet with aeration stone were randomised within treatments to minimise tank effect. Light intensity in experimental chambers during the period of daylight ranged from 12 to 18 lux at the water surface. The bottoms of the experimental chambers were covered with a 3 cm thick bed of sand and river gravel (1 – 5 cm diameter) providing shelter for trout. The sides of the experimental chambers were covered by black plastic bags, with an observation window in the middle of each experimental chamber.

Experimental protocol

Fish held in the experimental chambers were exposed for 40 days to 2x2 factorial density and familiarity experimental design. Groups assigned as familiar consisted of six fish originating from the same stream section and were kept together during the whole experiment. Groups assigned as unfamiliar initially consisted of six fish where each individual originated from different stream section. Density treatment was arranged by different bottom area of experimental chambers, with the large experimental chambers presenting a low density treatment and small experimental chambers presenting a high density treatment. This arrangement resulted in a population density of 95 individuals per m^2 in the high density treatment which is close the upper limit of 0 + brown trout densities reported as a natural (Elliott 1994; Brockmark at al. 2010). In total we had six replicate groups for each of four treatments; *i)* unfamiliar individuals kept under high density *ii)* unfamiliar individuals kept under low density *iii)* familiar individuals kept under high density *iv)* familiar individuals kept under low density (Fig. 1).

Before housed to the experimental chambers, fish were anesthetized (2-phenoxyethanol, 0.5 ml/l), and tagged with fluorescent visible implant elastomer tags (VIE; Northwest Marine

Technology Inc., WA, USA) to allow individual recognition. The VIE was injected subcutaneously on the left side of the fish on two locations; at the base of the adipose and the anal fin (Olsen & Vøllestad 2001). Thereafter, digital photographs of the fish were taken and individuals were sorted among the experimental chambers. Body length (fork length, FL; precision 0.1 mm) and damage of the caudal fin was measured from the photographs using the software ImageJ 1.46r (http://imagej.nih.gov/ij/).

Digital photographs of all individuals were subsequently taken during regular samplings every ten days at the end of each experimental round. Before the sampling all individuals were gently netted from the experimental chambers and anesthetized in the same way as during the initial tagging. Individuals were placed back to the experimental chambers after the photographs were taken. To maintain a low familiarity among fish in the unfamiliar treatment the individuals were swapped systematically between the groups with the same density treatment creating a completely new groups of unfamiliar individuals. Fish in the familiar treatment were placed back to their original experimental chambers after the sampling. Before the individuals were placed back the bottom substrate was shuffled in all experimental chambers in order to standardize the effect of environmental novelty for individuals. Fish were fed frozen chironomids larvae at a daily ration of 8% of mean fish wet weight. Food was dispersed evenly by a disposable pipette over the whole surface of experimental chamber every day at noon. Experimental chambers were controlled during the feeding for dead individuals and those were removed immediately. Decrease of the group size caused by mortality was balanced by proportional reduction of the experimental chamber to keep density of individuals constant. The size of experimental chambers was reduced by inserting a new plastic divider. In unfamiliar groups, dead individuals were replaced during the regular samplings by size-matched individuals with the same stream section of origin as the dead fish. In familiar groups where mortality occurred the tank area remained proportionally reduced until the end of experiment. We performed four samplings and swapping of individuals over 40 days of the experiment, creating four experimental rounds.

Emergence test

All experimental groups were tested in an emergence test to examine dispersal tendency of individuals within the groups. The emergence test was performed a four days after the last (the fourth) experimental round. The trial arenas consisted of three opaque rectangular plastic tanks (120 x 50 cm, water level 6 cm) with two gravel beds situated at the opposite narrow sides of the rectangular (20 x 50 cm). The gavel beds were divided by an open middle area without any structure and lined with white plastic film (80 x 50 cm). The white barren mid-section was assumed to present an unfavourable and stressful environment (Johnsson et al. 2000) while gravel beds were assumed to be perceived as shelters. One of the gravel beds was closed off from the rest of the trial arena with an opaque panel equipped by removable door (3,5 x 5 cm) and it was used as a starting chamber. Experimental groups were released to the starting chamber and after an acclimation period of 15 minutes the opaque door was hoisted and individuals were free to leave the chamber and explore the rest of the trial arena. The upper limit for the shelter emergence was 45 minutes. The tests were conducted in eight batches of three groups during one day from 8:30 until 19:00. Individuals were not fed for 24 hours before the trial start to standardize hunger levels. Trial arenas were cleaned and filled with fresh water before a new batch of fish was tested. Movement of individuals was recorded by digital video-cameras (Toshiba Camileo S20, Tokyo, Japan), which were mounted in the lab ceiling above the trial arenas. The elastomer tags were not visible on the recording; therefore only a time to emerge from shelter of the first, last and the largest individual was recorded. In addition, the position of the largest individual in the emergence sequence relative to other group members was recorded. The difference in size among individuals was significant enough to determine the largest individual in all but one group. When mortality occur after the fourth swapping (*i.e.* before the emergence test), individuals were not replaced in both familiar and even unfamiliar treatment. Consequently, the mergence test was performed with 12 familiar groups where four of them consist of 5 and eight of 6 individuals and 12 unfamiliar groups where one consist of 4 and eleven of 6 individuals.

Data handling and statistics

Growth of individuals was calculated as an absolute length increment between consecutive measurements at the beginning and the end of each experimental round, yielding a four measurements of growth for each individual.

$$SGR = (\ln(\textit{final fork length}) - \ln(\textit{initial fork length}))/$$

$$\textit{length of an experimental round}\ (10\ \textit{days}) * 100$$

Individual growth data were pooled for all experimental rounds and analysed using a linear mixed model. The initial model contained familiarity (two levels: unfamiliar and familiar) and density (two levels: low and high) treatment, size rank of individual within a group (six levels or less according to the actual group size), experimental round (four levels) and their interactions as fixed factors and individual ID and stream section of origin as random intercepts. To avoid the mistreatment of covariate interaction terms, non-significant interactions were removed from the model and the model was run again without them (Engqvist 2005). By a step-wise method selection from the highest non-significant interaction were eventually removed all interaction terms and the final model presented in the results contained only the main effects of fixed factors. Differences among categories of fixed factors were tested using Tukey's HSD post-hoc test.

Effects of density and familiarity treatments and size rank of individual on fin erosion were tested using Fisher's exact test of categorical bimodal variables (presence/absence of fin damage). In the cases where the fin of individual was eroded in previous rounds only an increase of the eroded fin area was evaluated as a presence of the fin damage.

Time of emergence of the first individual and delay between emergence of the first and last individual from the shelter was used as scores of a group dispersal tendency. We assumed that earlier emergence of the first individual from the shelter implies higher dispersal and that larger delay between the emergence of the first and last fish indicates low cohesive tendency of the group. Both variables were analysed using linear model, with density, familiarity and their interaction as fixed factors. Effects of density and familiarity treatment on position in the sequence of emergence of the largest individual relative to other group members was tested using Fisher's exact test for two levels (categorical variable: the largest individual emerging the shelter on the first or last position - 1, the largest individual emerging the shelter on an intermediate position – 0). Merging of the occasions when the largest individual left the shelter at intermediate positions in to one category and occasions when it emerged the firs or last to another category was used to increased statistical power of the test due to sample size (24 groups) which was low relative to the number of categories. Therefore this model tested, the strength of motivation and social influence of the dominant individual, but not its preference to leave or stay in the shelter.

Results

Effect of density and familiarity on growth

Growth of individuals held in the familiar groups was higher than growth of individuals in unfamiliar groups ($F_{1;131}$ = 4.38; p = 0.0384), but there was no overall effect of density treatment ($F_{1;131}$ = 0.02; p = 0.877; Fig. 2). Growth of individuals differed across the experimental rounds ($F_{3;406}$ = 11.26; p = $p < 0.001$). During the third experimental round was growth of familiar individuals held under the high density significantly higher than growth of unfamiliar individuals kept under the high density (Tukey-Kramer adj. p = 0.041), but there was no difference in growth among familiar and unfamiliar groups under the low density.

Growth was related to size rank of individual within the group ($F_{5;479}$ = 11.27; $p < 0.001$). Specifically, the largest individual grew faster than the rest of the group (Tukey-Kramer adj. $p < 0.001$), while differences in growth of other group members were not related to their size rank (Tukey-Kramer adj. $p > 0.05$; Fig. 3).

Fin damage and survival

There was difference among the treatments in proportion of individuals with eroded tail fin (Fisher's Exact Test $p < 0.001$). The proportion of individuals with the fin damage was 7.0 % in high density familiar, 13.8 % in high density unfamiliar, 1.4 % low density familiar and 12.0 % low density unfamiliar treatment. There was no relationship between the size rank and fin damage (Fisher's Exact Test p = 0.378). Survival was 92.36 % (133 out of 144 individuals survived) and no effect of density or familiarity treatment was observed. There was tendency for the individuals in the second and third size rank to show higher mortality than the rest of the group (Fig. 3).

Emergence test

There was no effect of density ($F_{1;21}$ = 0.05; p = 0.827) or familiarity ($F_{1;21}$ = 1.99; p = 0.172) treatment on time of emergence of the first group member. Delay between the time of emergence of the first and last individual was also not affected by density ($F_{1;21}$ = 0.34; p = 0.568) or familiarity ($F_{1;21}$ = 0.12; p = 0.728) treatment. The largest individuals in the familiar groups left the shelter either early and as the first (6 out of 12 groups) or late and as the last (5 out of 12 groups) from the whole group (Fig. 4). This pattern was significantly different from the unfamiliar groups, where no clear order was detected (Fisher's Exact Test p = 0.007). Density did not affect the propensity of the largest individuals to leave the shelter.

Discussion

This study shows that familiarity promotes growth of juvenile brown trout despite increasing population density. The lower tail fin erosion and higher growth rate observed in familiar groups also implies that aggression was lower among familiar individuals. Growth was closely related to the size rank of individual within the group with the superior performance of the largest (*i.e.* dominant) individuals of the group indicating the importance of individual's social status on growth. Dispersal of the group in the emergence test was not affected by familiarity or density treatment. However, the largest individuals in familiar groups left the shelter either as the first or the last ones, while there was no such pronounced pattern observed in unfamiliar groups.

The positive effect of familiarity on growth was the most striking in groups kept under high population density, while we found weak effect of familiarity in low density treatment. Interestingly, this results is similar to findings of a recent study (Fernandes et al. 2015) that growth of sibling groups of Atlantic salmon *Salmo salar* is increasing with population density, while there was a weak negative effect of density on growth in mixed groups. Since the amount of food per individual and habitat complexity in our study was similar in both density treatments, we suggest that growth was affected by aggression and social stress caused by proximity of competitors (Grant & Kramer 1990; Praw & Grant 1999). This interpretation was supported by higher fin damage among unfamiliar individuals which is an indirect indicator of aggression in salmonids (Cañon-Jones et al. 2011). In detail, the manipulation of population density *via* size of the holding tank has two ecologically relevant consequences; inter-individual distance decreases with decreasing tank size while food density increases with decreasing tank size. Size of the tank itself should further increase rate of the aggressive interactions, because brown trout initiate attacks faster in smaller tanks (Sundström et al. 2003). This manipulation therefore results in a "rich" but "crowded" habitat in the small experimental chambers and "poor" but "vacant" habitat in the large experimental chambers. Thus familiarity likely mitigated aggression-related social stress allowing individuals to utilize food better (Höjesjö et al. 1998; Griffiths et al. 2004) in the "rich" but "crowded" habitat of the small experimental chambers. In contrast, unfamiliar individuals were exposed to increased aggressive interactions and scramble competition, which at high density may reduce the overall feeding efficiency in the group, simply because individuals get in the way of each other (Ruxton 1995). The negligible effect of familiarity on growth in the low-density treatment contrasted with difference in the probability of fin damage which was much higher among unfamiliar individuals in the large experimental chambers. This implies that the effect of lower aggression among familiar individuals on growth was outweighed by other factors in the large experimental chambers. A possible reason is that in that the low density of food in the large experimental chambers emphasized importance of cognitive ability (Johnsson & Kjällman-Eriksson 2008) and alternative feeding tactics (Höjesjö et al. 2005) on growth. The relaxing effect of familiarity on interference competition might be of high importance even in under natural conditions, where individuals are often aggregated within a patchily distributed habitat in a small groups with a strong social hierarchy (Elliot 1990; Griffiths & Magurran 1997; Kurvers et al. 2013). In accordance, stability of a group has been shown to mitigate negative effect of increased population density on growth of juvenile brown trout in the wild Závorka et al. (*in press*). Group stability increases familiarity among individuals (Griffiths 2003) and thus this effect might be driven by processed described in our study.

The positive effect of familiarity on growth in present study was pronounced since the first experimental round. This may imply that individuals were able to recognise conspecifics from the same stream section before the familiarity within the experimental chambers was established. The kin selection hypothesis (Hamilton 1964; Fernandes et al. 2015) might provide an alternative explanation; as non-individual recognition based on general olfactory cues (Griffiths 2003) can result in lower aggression among siblings (Ward & Hart 2003). However, all individuals were caught within 500 m long stretch and thus all of them are likely siblings (Hansen et al. 1997). Additionally, in contrast to the kin selection hypothesis some studies have suggested that groups of siblings suffer from higher competition due to similarity of their phenotypes (Griffiths & Armstrong 2001; Greenberg et al. 2002).

Body size in animals is often positively correlated with their social status (Ward & Hart 2003; Bergeron et al. 2010). In our study, the size rank of an individual was positively correlated with their growth rate. This indicates that large individuals are able to utilise the competitive advantage of their size for social dominance even in familiar groups, which is consistent with some previous studies (Granroth-Wilding et al. 2013). In contrast, findings of Seppä et al. (2001) and Utne-Palm and Hart (2000) suggests that food sharing and growth rate in arctic charr *Salvenius alpinus* and threespine stickleback *Gasterosteus aculeatus* is more equal in familiar than in unfamiliar groups. Brown trout is, however, in general more territorial and aggressive than the latter species (Elliott 1994). The superior performance of the largest (i.e. dominant) fish contrasted with the growth of the second and third ranked individuals, which grew similarly as the other group members, but tended to suffer from higher mortality.

Similarly, previous studies on the giant kokopu *Galaxias argenteus* Hansen & Closs (2009) and brown trout Sloman et al. (2000) have reported that individuals which fail to become dominant (usually the second and the third in the size rank) are exposed to more aggression than the rest of the subordinate individuals.

Familiarity may also affect the inter-individual dependence of behavioural decisions within a group. During the emergence test we found that the dominant fish in familiar groups generally left the shelter either first or last whereas no such pattern was found in unfamiliar groups. Interestingly, dominants in familiar groups which left the shelter as the first did so early after they were free to leave, while dominants which preferred to stay in the shelter, left the shelter late or not at all (Fig. 4). We assumed here that the first or the last position in the sequence of individuals leaving the shelter likely represent a strong familiarity depended capability of the dominant individual to express a preference to explore the new environment or stay hidden in the shelter (Näslund et al. 2015). Our findings support the suggestion that more stabile social structure in familiar group allow dominant individuals make behavioural decision according to their individual preference. The mechanisms behind these effects need further study, but our results are generally in agreement with some studies on more social species, reviewed in Webster & Ward (2011), suggesting that behavioural decisions are affected by association between the social structure of animal groups and individual personality. Our findings are not entirely consistent with the hypothesis that large dominant individuals generally act more cautiously in risky situations than small subdominants due to their larger energy reserves and prior access to resources as has been suggested by de Laet (1985). Rather the decision seems to hinge on whether the dominant fish perceives the enclosed shelter to be more, or less, threatening than the open field of the trial arena (Näslund et al. 2015). Since the hunger level was controlled before the experiment, we suggest that the individual behavioural strategy (Adriaenssens & Johnsson 2013), rather than energetic stress might govern the choice of the dominant fish.

In summary, the present study showed that the mitigation of aggressive interactions by familiarity despite the increasing population density has a positive effect on growth rate of all individuals within the group. However, familiarity seemed to be especially advantageous for dominant individuals, which apart of the superior growth and were less constrained by other group members in behavioural decisions they made in the emergence test.

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Figure legend

Fig 1 Diagram of experimental protocol illustrating the stream section of origin and distribution of individuals among treatments during the first experimental round.

Fig 2 The boxplot present specific growth rate growth of juvenile brown trout across experimental treatments (HU - high density, unfamiliar; LU - low density, unfamiliar; HF - high density, familiar; LF - low density, familiar).

Fig 3 The boxplot present specific growth rate of individuals in relation to their the size rank, individuals in the first size rank grew significantly faster than the rest of the group ($p < 0.001$). Barplot below the boxplot shows frequencies of dead individuals in the size ranks across the whole experiment

Fig 4 Plot of the order in sequence and time of leaving the shelter in emergence test by the largest individual in group. Full circles present familiar and empty circles present unfamiliar groups

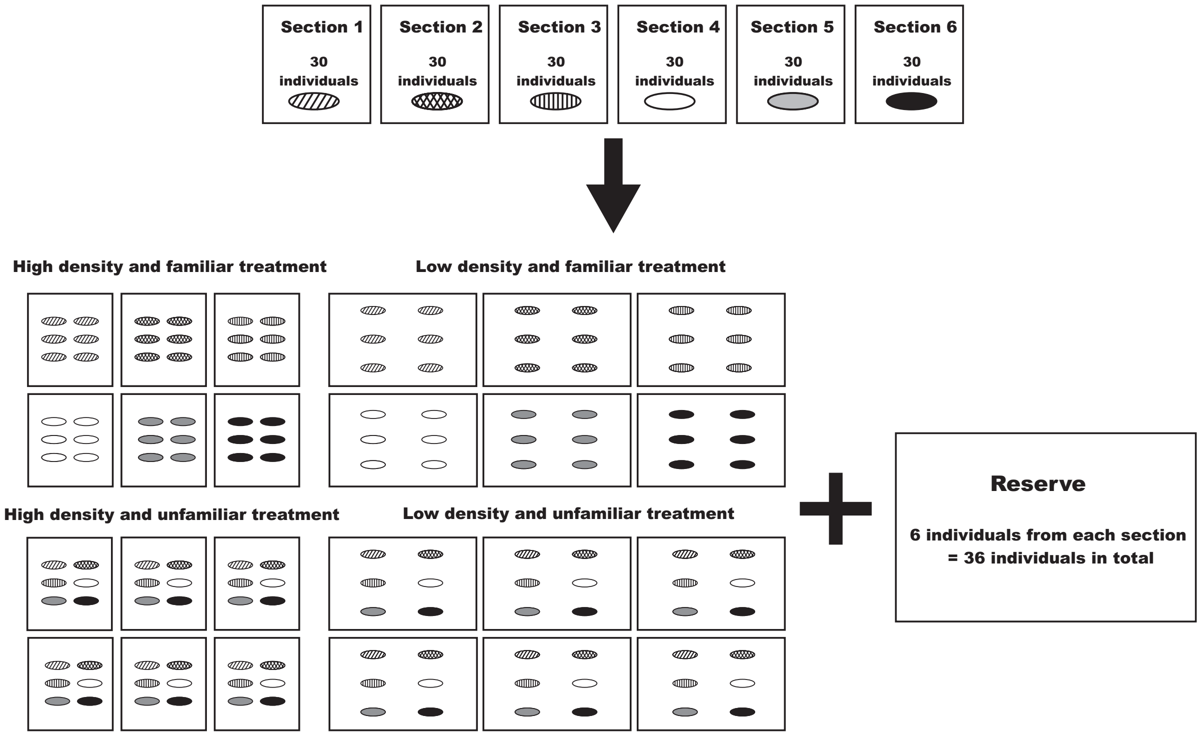
Section 1
30 individuals
Section 2
30 individuals
Section 3
30 individuals
Section 4
30 individuals
Section 5
30 individuals
Section 6
30 individuals
High density and familiar treatment
Low density and familiar treatment
High density and unfamiliar treatment
Low density and unfamiliar treatment
Reserve
6 individuals from each section
= 36 individuals in total

Figure 1

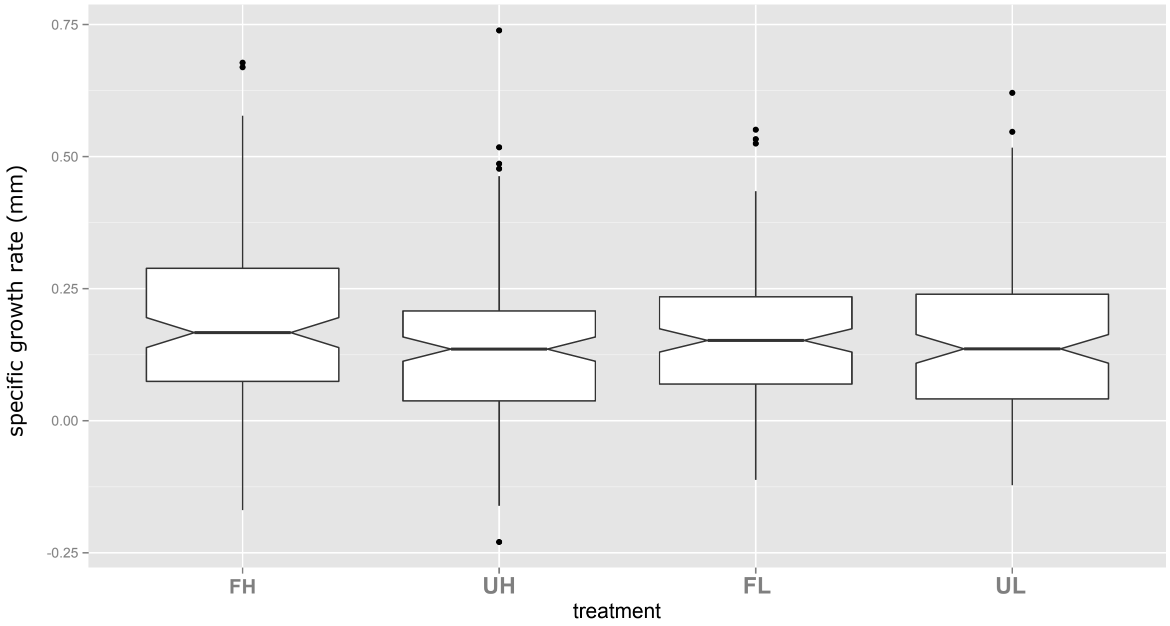
0.75
0.50
0.25
0.00
-0.25
specific growth rate (mm)
FH
UH
FL
UL
treatment

Figure 2

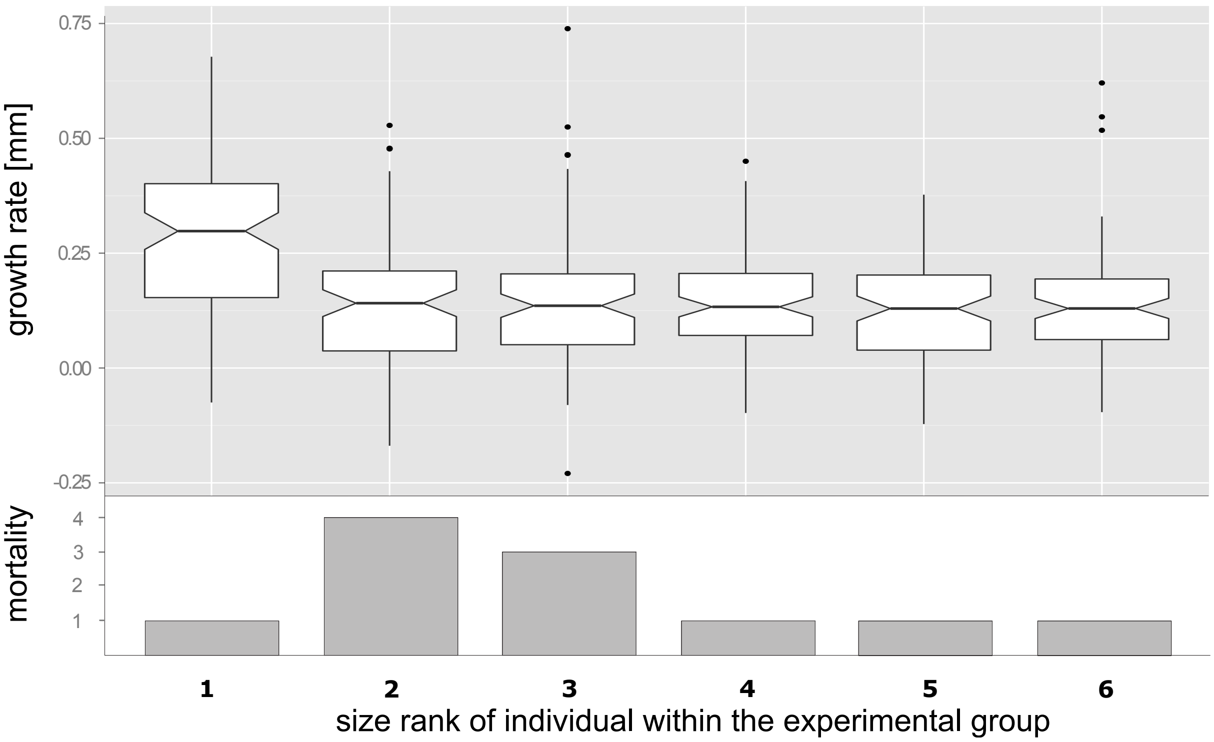
0.75
0.50
0.25
0.00
-0.25
growth rate [mm]
mortality
4
3
2
1
1
2
3
4
5
6
size rank of individual within the experimental group

Figure 3

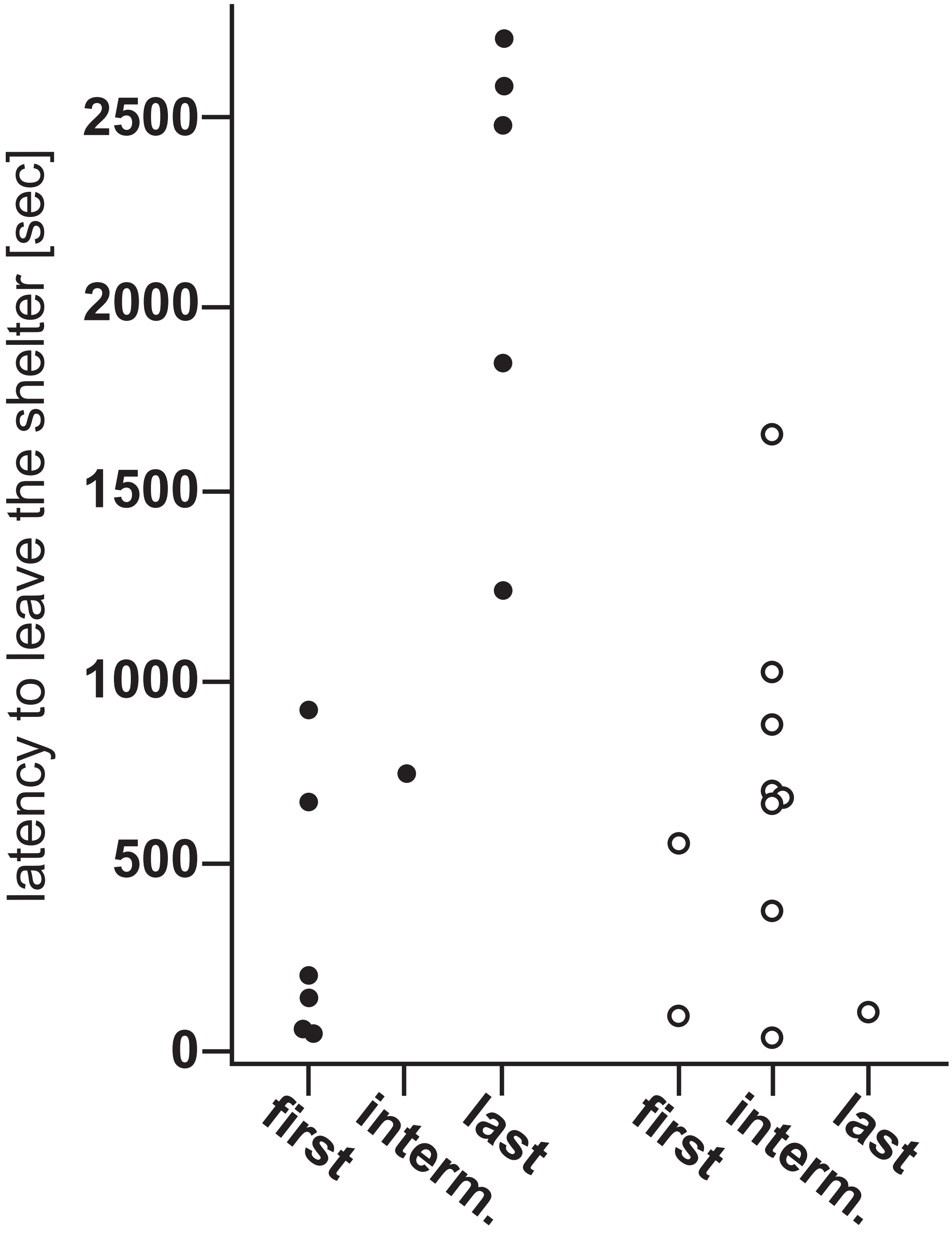
latency to leave the shelter [sec]
2500
2000
1500
1000
500
0
first
interm.
last
first
interm.
last
order to leave the shelter

Figure 4

PAPER III

Näslund J., Aldvén D. and Závorka L. (2015) Eggs from anadromous adults provide marine-derived nutrients to Atlantic salmon and brown trout parr prior to the onset of winter – observations from a Swedish coastal stream.

Eggs from anadromous adults provide marine-derived nutrients to Atlantic salmon and brown trout parr in late autumn – observations from a Swedish coastal stream

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Abstract

In this paper we demonstrate that eggs from anadromous salmonines can constitute a large proportion of the dietary intake of individual juvenile Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* during the spawning period in late autumn. The majority of fish older than one year residing on a spawning ground were found to have ingested eggs from spawning salmonines (*Salmo* sp.). Young-of-the-year brown trout were also able to consume eggs, at least down to a minimum fork length of 68 mm. The smallest Atlantic salmon found to have consumed eggs had a fork length of 79 mm. Larger fish were more likely to consume eggs, and consumed more eggs, than smaller individuals. The eggs of anadromous salmonines provide a rich source of marine-derived nutrients to the freshwater residing juveniles at the onset of winter. Our study highlights that these eggs may constitute an important energy input for juvenile salmonines in European anadromous populations, and could possibly influence winter survival rates and subsequent smoltification in the following spring.

Keywords: Salmonidae, Salmoninae, Oophagy, Egg cannibalism, Spawning, Europe

Introduction

Fish eggs are rich in energy and a valuable food source for many animal taxa, such as many species of fishes (Cummins and Wuycheck 1971, Smith and Reay 1991, Bunn et al. 2000, Fuiman et al. 2015). In comparison to most other actinopterygian fish, fishes belonging to the salmonid subfamily Salmoninae (salmon, trout and char) have relatively large and energy-rich eggs (Kamler 2005), which are commonly subject to predation, both from hetero- and conspecifics (e.g. White 1930, Greeley 1932, Maekawa 1983, Blanchfield and Ridgway 1999, Scheuerell et al. 2007, Garner et al. 2009, Aymes et al. 2010). For example, the North American Dolly Varden char *Salvelinus malma* utilize eggs deposited by heterospecific anadromous salmonines (Pacific salmon *Oncorhynchus* spp.) as one of their primary food sources, even though these eggs are available only at a limited time of the year (Denton et al. 2009, Armstrong and Bond 2013). As the anadromous Pacific salmon feed in the ocean prior to the spawning, their eggs constitute marine-derived nutrients (MDN). The importance of MDN in freshwater ecosystems is well established in North America, due to the fact that salmon migrating upriver from the Pacific coast are semelparous and consequently leave both eggs and their dead bodies as potential sources of nutrients (Bilby et al. 1996; Cederholm et al. 1999; Schindler et al. 2003). In contrast, salmonine MDN has attracted considerably less attention in Europe, most likely because the spawning migrations are less conspicuous and constitute of iteroparous species. A few investigations have been published and also suggest that salmonine MDN may play an important role for Atlanto-European stream ecosystems (Elliott et al. 1997; Lyle and Elliott 1998; Jonsson and Jonsson 2003; Williams et al. 2009). The spawning of the Atlanto-European anadromous salmonines, Atlantic salmon *Salmo salar*, brown trout *Salmo trutta*, and Arctic char *Salvelinus alpinus* occurs just prior to the onset of winter (Klemetsen et al. 2003). The offspring then resides in freshwater for a minimum period of one year, but usually for two or more years (Jonsson and Jonsson 2011). Thus, juvenile salmonines inhabiting streams and rivers could feed on eggs from anadromous adults to increase energy intake and winter survivability (Gende et al. 2002). Egg consumption by

European salmonines has previously been observed in Norway (Gravem 1981 [data presented also in Jonsson and Jonsson 2011]), Sweden (J. I. Johnsson, personal communication), Great Britain (McFarland 1925; Jones and King 1952), and France (Aymes et al. 2010). However, these observations have only been made on fish actively spawning, i.e. adult stream-resident fish or mature male parr. Information on egg consumption by juvenile fish remains scarce, especially with respect to the minimum size requirements for individuals consuming the large salmonine eggs. Small parr are most likely limited by their gape-size and thereby cannot ingest the eggs. A previous study has shown that Atlantic salmon parr (age 0+) were unable to cannibalize eggs, whereas 9.1% of age 1+ and 52% of age 2+ parr did so (Cunjak and Therrien 1998).

In this study, we investigated whether stream-resident individuals (mainly juveniles) of wild Atlantic salmon and brown trout consume eggs during the spawning season in late autumn. Furthermore, we also investigated the relationship between egg consumption and individual body size in order to determine the minimum size required for the consumption of eggs. Finally, we discuss possible implications of MDN, in the form of salmonine eggs, for the Atlanto-European salmonine populations.

Methods

The study site was a coastal stream (Bodeleån) located on the Swedish west coast (N 58° 19.745', E 11° 54.891'). The distance from the sea to the first definite barrier for migrating fish (i.e. stream length) was 1700 m. Downstream from the migration barrier the width of the stream was approximately 3 – 6 meters. Natural populations of anadromous Atlantic salmon and brown trout spawn in the stream during late autumn.

In 2013, we took an opportunistic stomach content sample from Atlantic salmon and brown trout parr (age 1+; excluding mature male parr) when collecting and tagging fish for an over-winter field study. On 29 October 2013, we caught 182 fish (37 Atlantic salmon; 145 brown trout) by electrofishing (LUGAB L-600, Lug AB, Luleå, Sweden; 200-300 V, ca. 1 A) from five 50 m sections of the river. These sections were randomly located from 530 to 1320 m upstream of the outlet. Some of these sections overlapped spawning areas and brown trout were observed spawning at the time of the sampling (personal observations), however it was not noted whether fish were caught in areas with ongoing spawning. Due to the high water flow we could not electrofish the central parts of the stream. The fish were brought to the university laboratory whereupon stomach contents were collected using non-lethal stomach flushing (as described below). Only the presence or absence of salmonine eggs and egg shells was noted.

Stomach contents were collected by flushing water into the stomach of the fish using a plastic disposable 7 ml Pasteur pipet (VWR International, Radnor, PA, USA). The pipette was inserted into the stomach via the oesophagus, and water was into the stomach while maintaining light pressure on the abdomen of the fish. This procedure forced the water containing the stomach contents to exit via the oesophagus. The procedure was repeated until only water was present in the material exiting the oesophagus (approximately 3-7 times per fish). The flushing method we employed was similar to the 'syringe flushing' method described by Meehan and Miller (1978). Stomach flushing methods have been shown to provide good quantitative results, although there is a possibility for underestimating the number food items as some content may be left in the stomach (Andreasson 1971; Meehan and Miller 1978). All fish were anaesthetised (2-phenoxyethanol, 0.5 ml $\cdot$ l^{-1}) prior to handling. Species were determined via a dichotomous key (Otterstrøm 1935). Individual fork length (FL; 1 mm precision) was measured directly prior to the stomach content sampling procedure. After stomach flushing, the fish were monitored in the laboratory for two days and no mortalities were observed. The fish were released back into the stream on 31 October 2013.

On 30 October 2014, a second sampling of parr was carried out from one of the major brown trout spawning grounds (700 – 800 m upstream the outlet). The fish (16 Atlantic salmon; 40 brown trout) were anaesthetised (Benzocaine, 0.5 ml$\cdot l^{-1}$) and stomach contents were sampled on site using the same procedure as described above. In this year, non-lethal sampling was chosen as a precautionary measure due to the relatively low number of smolts in this stream compared to previous years (personal observations).

We noted species, FL and whether the fish was a mature male parr with running milt. We collected stomach contents from a random subsample of 27 fish (10 Atlantic salmon; 17 brown trout), whereas we only noted the presence of eggs in the stomach for the remaining fish. Spawning adult brown trout were observed during the sampling and spent brown trout had been observed in the stream during a electrofishing-survey three days prior to the sampling (personal observations). The fish were released back into the stream after the sampling event.

Logistic regression models were constructed to investigate the effects of body size (i.e. fork length) on the probability of the presence of eggs in the stomachs of fish. In statistical analyses we used the data set from 2014, as these fish all had the opportunity to ingest eggs shortly prior to capture (i.e. they were caught in the spawning area where eggs were present), in contrast to the fish sampled in 2013. The relationship between body size and number of eggs found in the stomachs in 2014 was analysed using Spearman's rank correlation.

Results

In 2013, eggs or eggshells were present in the stomachs of 8% (3 out of 37) of sampled Atlantic salmon parr (FL range: 79 – 127 mm; mean ± SD: 91.9 ± 13.0 mm) and 20% (29 out of 145) of sampled brown trout (FL range: 78 – 140 mm; mean ± SD: 101.5 ± 12.2 mm). Frequencies of egg consumers in relation to body size are shown in Fig. 1a.

In 2014, when the fish sampled were residing on a spawning ground, eggs or eggshells were present in the stomach of the majority of fish larger than 80 mm (Fig 1b). The brown trout showed clear tri-modal distribution of fork length, which relates to different age cohorts (Fig 1b) (Weatherley 1972; Bohlin et al. 1996). The lower mode represent young-of-the-year (0+; FL range: 52-75 mm; mean ± SD: 66.1 ± 6.9 mm; N = 21), the middle mode represent age 1+ (FL range: 95-134 mm; mean ± SD: 114.8 ± 11.5 mm; N = 16), and the upper mode represent stream resident adult trout (older than 2 years; FL range: 173-176 mm; mean ± SD: 174.0 ± 1.7 mm; N = 3). Two individual young-of-the-year brown trout (FL: 68 and 72 mm) had eggs in their stomachs, demonstrating that it is possible for this cohort to consume eggs if they reach a large enough size prior to the spawning event. The majority of the 1+ group (88%) had eggs in their stomachs. All three stream resident trout that were sampled had also consumed eggs. The modality for Atlantic salmon, and thereby the assignment to year classes, was obscure due to the smaller sample size (FL range: 173-176 mm; mean ± SD: 98.1 ± 23.4 mm; N = 16). The smallest salmon found to consume eggs had a fork length of 79 mm, suggesting that 0+ salmon could also consume eggs. All mature male parr (n=7) were found to be Atlantic salmon (FL range: 87-130 mm; mean ± SD: 109 ± 17 mm) and all but one had consumed eggs (86%).

Out of all non-egg food objects collected from stomachs in 2014 (Table 1), only 6 % (n=10) were of comparable size or larger than salmonine eggs. Thus, it appears that eggs are the major food source at the investigated spawning ground, with respect to quantity and mass.

Logistic regressions demonstrate that the probability of finding eggs in the stomach of a fish (on the spawning ground) increases with fork length (Fig. 2). This relationship was significant for brown trout ($p < 0.001$), but not for Atlantic salmon where we only detected a trend ($0.1 > p > 0.05$). However, parameter estimates were similar for both species (for statistical details see Fig. 2), so the lack of significance for Atlantic salmon may be due to the lower sample size and the general lack of smaller individuals (Fig. 1). The majority of fish larger than 80 mm FL (corresponding to fish at age 1+, or older; see Fig. 1) were found to have consumed eggs.

Analyses of stomach content samples clearly demonstrate that eggs are the predominant food objects on the spawning grounds (for fish large enough to eat them) (Table 1). Generally, larger fish had higher numbers of eggs present in their stomach (Spearman's rank correlation:

Atlantic salmon: $\varrho = 0.65$, $P = 0.044$, N = 10; brown trout: $\varrho = 0.67$, $P = 0.003$, N = 17). For quantitative data on other food objects present in the stomach of sampled fish, refer to Table 1.

Discussion

Both in 2013 and 2014, we show that Atlantic salmon and brown trout parr consume eggs in a Swedish coastal stream. This clearly demonstrates that MDN are utilized as an energy source in this stream. Since many spawners of *Salmo* spp. return to sea after spawning, the eggs may constitute a great potential energy source for the stream ecosystem, even when compared to the amount of carcasses left in the river (Jonsson and Jonsson 2003). The eggs may be of a higher relative importance in the Atlantic river ecosystems when compared to Pacific river ecosystems, where many of the anadromous salmonine species invariably die after spawning. Furthermore, juvenile salmonines can receive MDN indirectly from the production stemming from carcasses of dead spawners, but they exhibit a direct MDN benefit when eating the eggs (Gende et al. 2002; Denton et al. 2009; Williams et al. 2009). Egg consumption coincides with a period (late autumn) where high-energy reserves most likely influences winter survivability (Finstad et al. 2004; Huss et al. 2008).

Since we observed brown trout spawning at our study sites and our sampling period directly coincided with the well-documented spawning period of brown trout on the Swedish west coast (Degerman et al. 2001), we conclude that the eggs present in the stomachs of the fish are most likely from brown trout. However, there is no obvious reason to doubt that the salmonine juveniles in the stream also consume eggs from the spawning Atlantic salmon. Egg consumption appeared lower in 2013 when compared to 2014, however, this is most likely attributable to the fact that we sampled fish from areas were no spawning was ongoing in 2013 compared to only sampling in a spawning site in 2014. Even though the fish are free to move within the stream, the fish caught outside spawning sites in 2013 may not have had the opportunity to eat eggs within a couple of days of the sampling period, which could explain why no eggs were present in their stomachs. Unfortunately we were not able to differentiate between spawning grounds and other areas whilst collecting samples in 2013, which makes it impossible to investigate the relationship between the presence of eggs in the stomach and the location of the fish in the stream during this year. Nevertheless, given that the stream sections sampled in 2013 were randomly distributed in relation to spawning sites, the lower frequency of egg-eating individuals suggest that the importance of eggs as a food source may vary among individuals. Further investigations on the movements of immature salmonine parr through different stream sections during spawning may help explain the importance of eggs as a food source for these fish. Active migration of immature parr towards spawning grounds would indicate that eggs are an ecologically important food source.

The smallest brown trout found to have ingested eggs was 68 mm, whilst the smallest Atlantic salmon was 79 mm. Atlantic salmon has a smaller mouth in relation to body length than brown trout, thus the minimum size for egg consumption is most likely smaller for trout than for salmon, as our direct observations suggest. However, the parameter estimates of logistic regressions indicate that Atlantic salmon have smaller minimum size, but this is likely due to very few small sized Atlantic salmon parr being captured which makes the estimates imprecise for this species. The observed minimum sizes are definitely within the limits of sizes attainable by 0+ salmon and trout (e.g. Bohlin et al. 1996), which can also be observed in the size distribution found in our study. Thus, the size threshold for eating eggs could be one of the driving forces for rapid growth of 0+ fish in summer and early autumn. Potentially, becoming large enough to consume eggs in the first year of life may lead to increased winter performance (Huss et al. 2006) and further competitive advantage the coming year. A late autumn energy boost may also allow early smoltification one year before the typical age of seaward migration (i.e. at age 1+), which is seen in a small proportion of the fish in Swedish western coastal streams (Bohlin et al. 1996).

We found that all three stream resident brown trout ate eggs. These adult fish were most likely participating in the spawning activity (Höjesjö et al. 2007). This observation is in concordance with a study by Aymes et al. (2010), which showed that spawning brown trout also consume eggs. High frequency of egg eating by stream resident trout has also been observed in a nearby stream (Jörlandaån [N 57°58.851', E 11°48.018']; Jörgen I. Johnsson, personal communication).

The mature male parr sampled in this study consisted only of Atlantic salmon and the majority of these fish ate eggs, which is consistent with observations by Jones and King (1952). The reason why only mature parr of salmon were found in our study remains unclear, as brown trout typically have a relatively high frequency of maturation in the parr stage in the Swedish coastal streams (Dellefors and Faremo 1988). Mature trout parr could have been spent with no running milt at the time of the sampling and thus remained undetected or they may have been located at sites where electrofishing was less efficient.

Egg predation raises several interesting questions related to the biology of Atlantic salmon and brown trout. The level of predation could for instance affect inter-population differences in selection on egg size, as larger eggs would be less vulnerable due to gape-limitation of smaller potential predators (refer to Fleming 1996 for other explanations of large egg size in salmonines). However, if the main strategy is to eat stray eggs, then egg-size selection may be absent, as stray eggs are most likely not going to survive, regardless of being predated or not. In the present study we cannot determine whether the predators actively consume eggs in the nest or if they consume stray eggs, however, both types of predation have been previously observed in a French population of brown trout (Aymes et al. 2010). Active egg predation in the nest could be associated with substantial risks as both females and males defend the nest against intruders (Fleming 1998). A study by Tentelier et al. (2011) even suggest that male brown trout exhibit short term parental care, as they appear to temporarily stay at the nest site to prevent the newly laid eggs from being cannibalised. Furthermore, mature male parr and stream resident males are known to be at risk of serious injury from larger anadromous males when attempting to mate with females (Fleming 1996). Speculatively, parr approaching the size required for egg ingestion may need to access the nest shortly after the spawning event in order to reach the eggs prior to 'water hardening', which increases the diameter of the eggs (Foote and Brown 1998). Thereby, the smallest egg-eating parr may be more exposed to aggression from spawners and egg predation may constitute a 'high risk – high gain' strategy for these fish. In order to investigate this hypothesis, we suggest that future studies should compare the growth and mortality of parr residing on spawning grounds with parr residing outside these areas.

The importance of egg predation for over-winter growth and survival, as well as for the timing of smoltification in the coming spring also warrants further investigation. Egg consumption has been suggested to have positive effects on these parameters in Pacific salmonines, and this may also be true in Atlanto-European salmonines (Gende et al. 2002). In European perch *Perca fluviatilis* high growth rates in autumn can reduce over-winter mortality as a consequence of a positive growth-dependent increase in allocation to energy reserves (Huss et al. 2008). However, in addition to MDN, the eggs may carry lipophilic toxic and endocrine disrupting compounds from the marine environment that may negatively affect the juveniles when consumed (see e.g. Niimi 1983; Sarica et al. 2004). Furthermore, a growth-mortality trade-off may be connected to egg consumption, caused by nest-defending adults. Previous studies in Europe, including ours, suggest that the rate of egg consumption can be high and that eggs may be a major food source during late autumn (Gravem 1981; Aymes et al. 2010). Thus, egg consumption could potentially be an important aspect of growth and development for juvenile salmonines and deserves more attention in the future research on European anadromous salmonine populations.

In conclusion, we present evidence for egg consumption by juvenile Atlanto-European salmonines in late autumn. Furthermore, we show that fish as young as age 0+ can consume eggs, provided that their growth during the summer period was high enough to reach a size of

70-80 mm FL. These findings present opportunities for future research on the importance and consequences of MDN in European coastal stream ecosystems, particularly regarding the performance of juvenile salmonines. Further investigations into this area may aid in the conservation and management of salmonine populations.

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Figure captions

Figure 1. Histograms of fish with and without eggs (empty egg shells were counted as eggs) present in the stomach during the survey years of 2013 (A; random sites in the stream surveyed) and 2014 (B; only spawning ground surveyed). Note the different scales of the x-axes.

Figure 2. Predicted probability of the presence of eggs (including empty egg shells) in the stomach of Atlantic salmon and brown trout on a spawning ground in the Bodeleån stream (2014). Parameter estimates (*B*) of the logistic regression, with standard errors (s.e.), are presented in the table along with statistics.

Table 1. Number of food objects found in flushed stomach contents of the individuals sampled in 2014 at the spawning ground. AS = Atlantic salmon; BT = brown trout; Unid. = unidentified species.

Species:	AS	AS	AS	AS	AS	AS	AS	AS	AS	AS	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
Fork length (mm):	79	87	88	92	104	117	117	121	129	130	68	73	75	75	95	100	103	105	113	120	120	125	128	130	173	173	176
Eggs		2		5		9		6	7	8	2				4	9		9		6	9	6		6	19	14	5
Egg shells		2		5	2	2		19	2	14	3				4	3		2		10	2	3	1	4	6	4	10
Ephemeroptera			4		1										1	2			1			1			1		
Plecoptera					1									1								3					
Trichoptera		2	5					1			1		1			1	2		2			3					1
Chironomidae					1	1		1	1				1	1		2		1	1	1		2				3	2
Simuliidae		1	4																								
Other Diptera larvae																3	2				1	1					
Unid. insect (aqat.)				3				2			1			3	2	7		2	1	4	1		1		1		
Unid. insect (terrest.)				1							1					1											
Asellus aquaticus			1		1						1		4				1							1			
Coeloptera (adult)											1																
Coeloptera (larvae)																				1							
Enchytraeidae				4	1	1			1	1				1	3	4	2	3	2		6	1				1	1
Lumbricidae														2		1						1					
Hirudinea													1														
Ancylidae		1	13		1																						
Myriapoda				1					2		1						1										
Unid. invert.			1			1														1	1						
Total eggs/egg shells:	0	4	0	10	2	11	0	25	9	22	5	0	0	0	8	12	0	11	0	16	11	9	1	10	25	18	15
Total objects:	0	8	28	19	8	14	0	29	13	23	11	0	7	8	14	33	8	17	7	23	20	21	2	11	27	22	19
% egg/egg shells	-	50	0	53	25	79	-	86	69	96	45	-	0	0	57	36	0	65	0	70	55	43	50	91	93	82	79

Figure 1

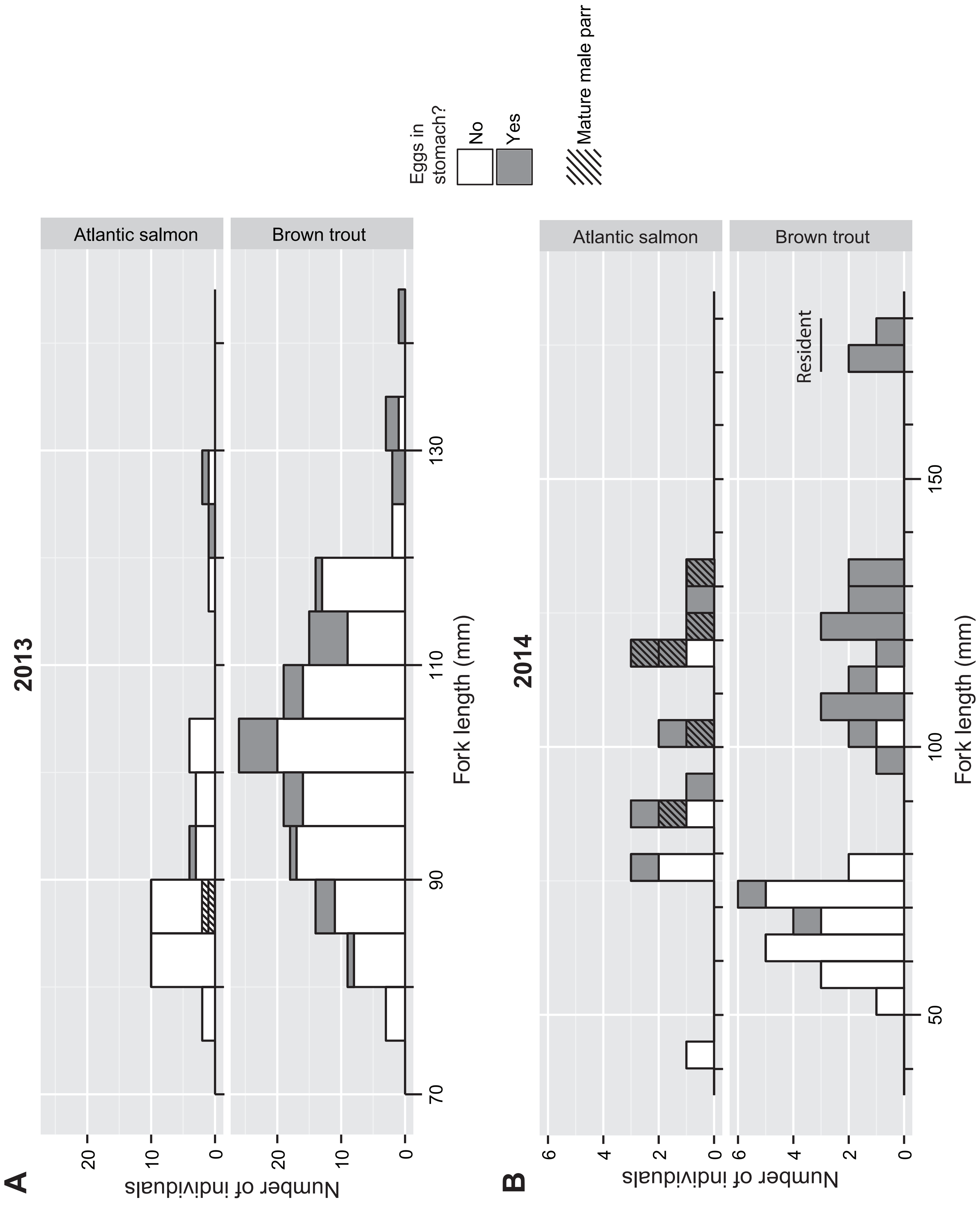
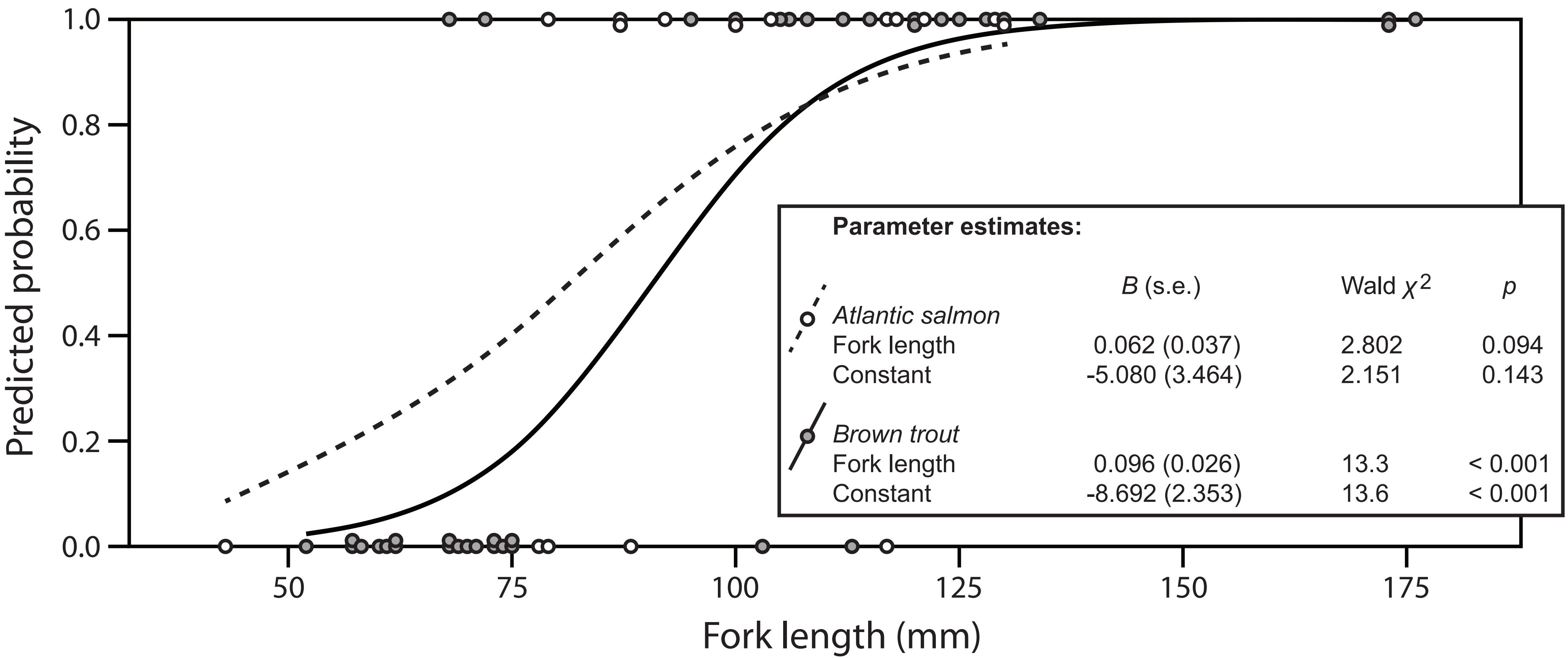

A
2013
Atlantic salmon
Brown trout
Number of individuals
20
10
0
70
90
110
130
Fork length (mm)
Eggs in stomach?
No
Yes
Mature male parr
B
2014
Resident
6
4
2
0
50
100
150

Figure 2



Parameter estimates:

	B (s.e.)	Wald χ^2	*p*
Atlantic salmon			
Fork length	0.062 (0.037)	2.802	0.094
Constant	-5.080 (3.464)	2.151	0.143
Brown trout			
Fork length	0.096 (0.026)	13.3	< 0.001
Constant	-8.692 (2.353)	13.6	< 0.001

PAPER IV

Závorka L., Aldvén D., Näslund J., Höjesjö J. and Johnsson J. (2015) The fluctuating cost of high activity – an extension of the Pace of Life Syndrom hypothesis.

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Original Article

Linking lab activity with growth and movement in the wild: explaining pace-of-life in a trout stream

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Theory suggests that consistent individual differences in activity are linked to life history where high activity is associated with rapid growth, high dispersal tendency, and low survival (the pace-of-life syndrome hypothesis). We addressed this influential hypothesis by combining behavioral studies with fine-scale positional scoring in nature, estimating how individual movement strategies in brown trout (*Salmo trutta*) associate with fitness correlates (growth and survival) in the wild. Initial dispersal in the wild was positively related to the laboratory activity. Moreover, the growth of individuals with high laboratory activity decreased with increasing home range size, whereas the growth of individuals with lower laboratory activity increased slightly with increasing home range size. Survival in the wild was not associated with laboratory activity. Our results do not support the original pace-of-life syndrome hypothesis. As an alternative explanation, we suggest that the growth of individuals adopting a high-activity strategy is more sensitive to variation in resource abundance (indicated by home range size) than the fitness individuals adopting a more passive strategy.

Key words: **behavior repeatability, dispersal, exploration, growth rate, natural selection, survival.**

INTRODUCTION

Movements, fine-scale or long distance, allow an individual to switch habitats and to avoid unfavorable conditions (Bowler and Benton 2005; Brönmark et al. 2008). The decision to move should depend on individual-specific trade-offs between the fitness benefits and costs associated with movement (Dingemanse and de Goede 2004; Matthysen 2005; Hanski et al. 2006; Dingle and Drake 2007; Cote et al. 2010). Accordingly, within-population variability in movements has been described for a wide range of animal taxa (Jonsson and Jonsson 1993; Ball et al. 2001; Attisano et al. 2013). This behavior often differ consistently across individuals within a population from fine-scale movements often measured as behavioral traits in animal personality research (i.e., activity and exploration; Réale et al. 2007) to large-scale movement strategies in the wild linked to home range (John-Alder et al. 2009) and seasonal migration (Ball et al. 2001).

The ecological significance of consistent movement strategies is especially striking in species where such strategies are tightly linked to life history (Dixon et al. 1993; Musiani et al. 2007). Brown trout (*Salmo trutta* L.) is a highly plastic species showing a range of ecological adaptations including 3 basic life histories associated with movement: stream dwelling, lake migratory, and sea migratory (Klemetsen et al. 2003), which result from different trade-offs between reproduction, survival, and growth (Roff 1992; Hendry et al. 2004). Reproduction and survival in brown trout, as in other organisms with indeterminate growth, are positively correlated with body size (Elliott 1994; Kingsolver and Huey 2008, but see Carlson et al. 2008). Thus, growth is an important fitness correlate that can be used to compare the success of alternative strategies at the juvenile stage (Stearns 1976).

Population density has been shown to affect growth rate, survival, and movement in salmonids and in other territorial species (Matthysen 2005; Einum et al. 2006). In high-density areas, individuals are facing a choice where they can either stay at the cost of reduced growth rate or move to another area with potentially better growth conditions (Einum et al. 2006). However,

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movement can also induce substantial costs, for example, energetic expenditure and increased risk of predation (Yoder et al. 2004).

The pace-of-life syndrome (POLS) concept (Réale et al. 2010) suggests that consistent differences in behavior and life-history traits can coevolve forming stable associations. According to this influential hypothesis, high activity and boldness or exploration is consistently associated with rapid growth and high mortality, whereas low activity and shyness or neophobia is associated with slow growth and low mortality (Biro et al. 2004, 2006; Stamps 2007; Cole and Quinn 2014). The POLS hypothesis has been supported by a number of studies conducted under captive conditions and/or with domesticated species (Biro and Stamps 2008; Adriaenssens and Johnsson 2009; Mittelbach et al. 2014). However, studies conducted under natural conditions have repeatedly failed to support the hypothesis (Dingemanse et al. 2004; Adriaenssens and Johnsson 2011, 2013; but see Smith and Blumstein 2008), suggesting that links between behavior and life-history traits are more variable in natural environments characterized by fluctuating resource abundance and competition levels (Adriaenssens and Johnsson 2009; Réale et al. 2010).

As indicated by the discussion above, the ecological and evolutionary significance of interindividual variation in activity and associated behavioral traits (boldness, exploration) is still poorly understood (Réale et al. 2007). Although laboratory scores of behavior often show some repeatability over time (Bell et al. 2009), it remains unclear to what extent behavioral variation carries over between different contexts. Indeed, the critical need for cross-context validation of behavior in both laboratory and natural conditions has recently been highlighted (Niemelä and Dingemanse 2014). Previous studies addressing associations between activity and life-history traits have used indirect measurements of activity and boldness like catchability to a net or a trap (Réale and Festa-Bianchet 2003; Biro et al. 2004, 2006) or open-field test (Murphy et al. 1994; Adriaenssens and Johnsson 2011, 2013), whereas detailed measurements of individual activity and its association with movement in the wild have been scarcer (Niemelä and Dingemanse 2014). This is likely due to limitations in methods available for animal positioning in the wild. Traditional technologies available for animal positioning (i.e., telemetry) are limited, as the size of the transmitter dictates the minimal body size of the focal animal (Jepsen et al. 2005). However, telemetry using passive integrated transponder (PIT) tags is a novel, effective, and low-invasive method for animal identification and tracking.

Here, we combine laboratory behavioral scoring with PIT-tag telemetry in the wild using juvenile brown trout as a model to address predictions from the pace-of-life-syndrome hypothesis via the following questions: 1) To which extent are interindividual differences in activity, measured in a laboratory open-field test, associated with a) initial dispersal in an unfamiliar stream section and b) home range size in the stream? 2) Are differences in laboratory activity associated with growth and survival in the wild in concordance with the POLS hypothesis? 3) How are these associations influenced by population density?

METHODS

Fish collection

In May 2013, 200 one-year-old brown trout (mean ± standard error [SE]: 78.10 ± 0.64 mm; range: 56–107 mm) were caught by electrofishing (Bohlin et al. 1989) in a 250-m-long section of Jörlandaån, a small coastal stream in western Sweden (57°58′N, 11°56′E) (Figure 1). In the laboratory, fish were housed in 3 holding tanks and starved for 1 day before tagging to get standardized initial measurements of the body size. The holding tank provided shelter (rocks and plastic plants) and fresh water from a flow-through filtration system (flow rate: 2 L min^{-1}). Photoperiod followed natural light cycles and temperature was kept at 11–13 °C throughout the experiment. After the acclimatization, trout were anesthetized (2-phenoxyethanol; 0.5 mL L^{-1}), and measurements of fork length (distance from the tip of the snout to the end of the central caudal fin ray) and body weight were taken, followed by tagging with 12-mm PIT tags (HDX ISO 11784/11785, Oregon RFID, Portland, OR) into the body cavity. Tagged fish were subsequently divided among 18 holding tanks (30 L, 30 × 32 × 34 cm, 11–12 individuals per tank) and left to recover for 5 days before behavioral scoring. Individuals were fed ad lib with Chironomidae larvae during this period.

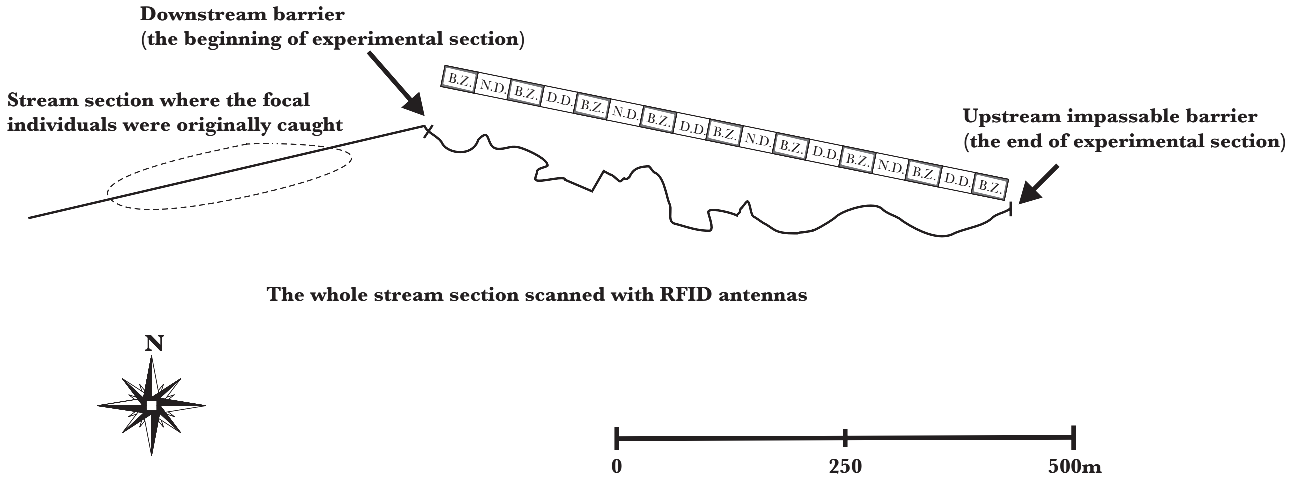

Figure 1
The experimental section of stream Jörlandaån. The rectangle illustrates the distribution of experimental subsections with manipulated population density. B.Z.: buffering zone; N.D.: subsection with normal population density; D.D. subsection with doubled density.

Behavioral scoring

Each fish was individually scored for swimming activity in an open-field test. The open-field trials were conducted in barren white rectangular plastic tanks (61 × 45 cm, water level 10 cm), positioned underneath a dim fluorescent light tube and a camera (Logitech webcam C120, Romanel-sur-Morges, Switzerland).

The fish were not fed for 24 h prior to the trials in order to standardize hunger levels of individuals in trial. When subjected to the trial, fish were gently netted from the holding tank and placed individually into trial tanks. Individual activity was scored for 30 min, following the first swimming movement. The trial tanks were divided by a grid of 20 equal-sized squares. Laboratory activity scores were derived from the number of crossings between squares, where each crossing represents a complete passage by an individual over the borderline into an adjacent square. After the scoring, all fish were scanned for individual PIT number and placed back into their holding tank; trial tanks were cleaned and filled with fresh water before a new batch of fish was entered. Trials were performed during 3 consecutive days from 8:30 AM until 7:00 PM under similar light and temperature conditions in order to standardize measured activity scores. There was no effect of day time on measured activity scores (Spearman's $\rho = -0.09$; $P = 0.193$). No mortality and no tag losses were observed during the laboratory period of the experiment. Fish activity was only scored once due to time constraints, as previous studies have found swimming activity to be repeatable over time in brown trout (repeatability in Adriaenssens and Johnsson 2013 was $R = 0.449$; $P = 0.006$).

Density manipulation

Following behavioral scoring, individuals were divided into a low-activity group and a high-activity group according to their laboratory activity score (Figure 2) and sorted in 8 release groups each containing 12 active and 12 passive individuals. The distribution of individuals was pseudo-randomized within the low- and high-activity groups, consequently each release group contained individuals with relatively high and low scores. Eight individuals with intermediate scores remained after the sorting and were removed from the experiment. The removal of surplus individuals resulted in a final number of 192 focal individuals (96 active and 96 passive individuals) that were released into the experimental stream section.

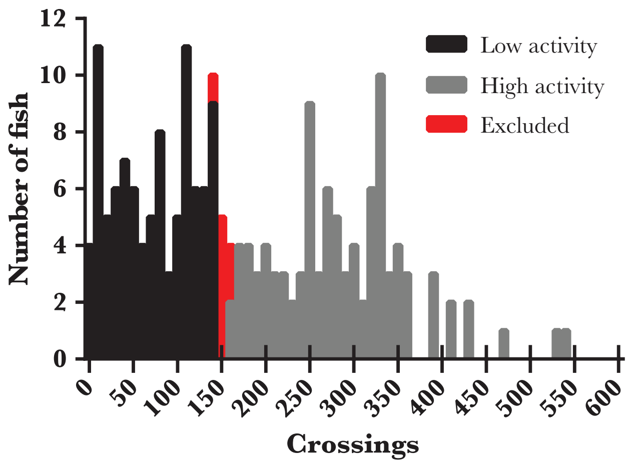

Figure 2
Distribution of activity scores of juvenile brown trout (activity scores were equal to the number of crossings between squares) in laboratory open-field test.

Each release group of focal individuals was put (on May 31) into one of eight 30-m-long experimental stream subsections in river Jörlandaån with manipulated trout density, either normal or doubled density. In subsections with normal density treatment, 24 resident age-1 trout were removed and replaced by 24 focal individuals. In the double density treatment, 24 resident age-1 trout were removed and replaced by 24 focal individuals and 24 introduced individuals, caught at least 200 m downstream from the used subsection (Figure 1). Removal of wild residents in experimental subsections was made to reduce prior residency effects in high-density subsections (Johnsson et al. 1999). Surplus individuals (8 fish) were released downstream the experimental section approximately 250 m below the barrier (Figure 1) in order to limit their homing migration back to the manipulated experimental stream section. All experimental subsections were separated from each other by 50-m-long buffering zones, which were supposed to be beyond the distance of regular movement range for juvenile brown trout (Bridcut and Giller 1993; Höjesjö et al. 2014). The experimental section was surrounded and shaded by deciduous (mainly alder) and spruce trees. The stream holds a population of sea migratory brown trout, which is the most abundant fish species (>95% by biomass); other species are European minnow *Phoxinus phoxinus* and European eel *Anguilla anguilla*. In addition to adult resident trout and eel, grey heron *Ardea cinerea* and American mink *Neovison vison* were predators observed to occur in the experimental area. The downstream limit of the experimental section was defined by a 0.25-m-high artificial weir. Movements upstream were hindered by a 1-m-high waterfall, an impassable obstacle for juvenile trout during the low water flow of the summer season. All fish appeared to be in good condition at the release.

Tracking and recapture

A longitudinal positioning of focal individuals was performed during the 3 summer months (June–August), using portable PIT-tag antennas (Oregon RFID). The first scanning took place 4 days after release, followed by biweekly scans, and the last scanning was performed 1 day before fish recapture on 3 September 2013. As individuals were able to move downstream from the experimental section; a stream section of 450 m below the lower weir was included in the scanning (Figure 1). Tracking was then conducted from this downstream point and upstream to the waterfall at the end of the experimental section, a total length 1400 m. Each observer was equipped with a GPS (eTrex Garmin, Olathe, KA), which automatically saved the position of the observer. Geographical positioning data of individual fish were obtained by synchronizing time stamps from the GPS and the RFID data logs. The repeatability and precision of positioning during the experiment was controlled against 3 fixed reference positions situated at the start, in the middle, and at the end of the scanned stream section (the standard deviation among scannings against these reference points was 2.4 m suggesting a good precision of positioning).

During recapture, on September 4, focal fish were positioned with the PIT-tag antenna and subsequently caught by electric fishing. When the focal fish were not attracted to the electrofishing wand, we tried to displace the individual out from the shelter by moving the bottom substrate. Individuals that did not respond to the electrofishing and kept the position even after the disturbance of the substrate were considered being dead (i.e., PIT tag was lying on the bottom). All recaptured fish (70 individuals) were anesthetized

(2-phenoxyethanol), PIT scanned, and measured for mass and body length. After the recovery, all individuals were released back to the place where they were caught.

Data handling and statistics

Fish movement in the field was measured as a longitudinal distance between 2 successive fish positions following the middle of the stream channel (i.e., meandering of the stream was taken in to account while diagonal movements within the channel were ignored). The distance from the point of release to the position of first scanning was defined as initial dispersal. Home range size was estimated as the distance between the 2 most distant positions where the individual was detected during the study, excluding the initial dispersal. This is a common method used for estimating home range along the longitudinal gradient of the stream (Hodder et al. 2007). These 2 variables represented movement strategies of individuals in the wild. One of the recaptured individuals was detected only once after the initial dispersal (i.e., home range size could not be estimated) and was therefore excluded from analysis. In contrast to our expectations, initial dispersal of the majority of individuals exceeded the distance between the experimental sections that eroded the original experimental setup of manipulated population density. Consequently, we tested for the effect of population density only in relation to initial dispersal of individuals.

Initial dispersal was analyzed using gamma regression (log-link function), which is suitable for strictly positive continuous data with positive skew (Hardin and Hilbe 2007). The model contained laboratory activity, initial density (2 levels: high and low density), their interaction, and initial weight as independent variables. Nonsignificant interaction between laboratory activity and initial density was removed from the model, and we reported results for the model without the interaction. The model contained all individuals detected during the initial scanning, 4 days after the release. To graphically investigate relationships between activity in the lab and initial distance moved in the stream, we plotted a Loess line (Epanechnikov kernel with 50% of points to fit) through a scatter plot of all data points.

Home range size was analyzed using gamma regression (log-link function), with the model containing laboratory activity and initial weight as independent variables. The model contained all individuals recaptured at the end of the experiment.

Survival was analyzed based on the recapture rates at the end of the experiment (on September 4). Individuals that were detected by PIT-tag antenna and caught by electrofishing gear were referred to as being alive, individuals detected by PIT-tag antenna but not caught referred to as dead, and undetected individuals were referred to as nondetected. There were 3 individuals referred as alive, which were not caught by the electrofishing gear, but they were displaced several meters upstream by the substrate disturbance. To investigate differences in laboratory activity among alive, dead, and nondetected individuals, we used linear model with status of individual at the final scanning (alive, dead, or nondetected) as an independent variable. Variance of data among the groups was homogenous (Levene's test: $P = 0.640$), and data were not normally distributed (Figure 2); however, model was robust against this assumption as the distribution of dependent variable did not differ among the groups (Schmider et al. 2010). Density treatment was not included in the analysis because many fish left experimental subsection shortly after release. The relationship between laboratory activity and the frequency of detection during the study was tested by nonparametric Spearman's rank correlation tests.

Individual growth was evaluated by specific growth rate (SGR) calculated as:

$$\mathit{Observed\ SGR} = \frac{\ln(\mathit{final\ wet\ body\ weight}) - \ln(\mathit{initial\ wet\ body\ weight})}{\mathit{time\ between\ measurements}\,(100\,\mathit{days})} \times 100 \tag{1}$$

The influences of home range and laboratory activity on specific growth rate were analyzed using all recaptured fish. To analyze growth rate, we first calculated deviation from expected specific growth rate based on initial size. Specific growth rate was negatively related to initial length, as expected (Brett 1979). Therefore, we used the parameters from a linear regression (slope and intercept) on the pooled data of all recaptured fish to calculate expected growth rate:

$$\mathit{Expected\ SGR} = 1.54 - 0.0126 \times \mathit{initial\ fork\ length} \tag{2}$$

Deviation from expected growth rate was calculated as:

$$\mathit{Deviation} = \mathit{observed\ SGR} - \mathit{expected\ SGR} \tag{3}$$

The deviation from expected growth rate was analyzed using a linear model. The linear model included laboratory activity, home range size, and their interaction as continuous independent variables. Diagnosis of the initial model indicated presence of one outlying value (Cook's $D > 1$ for 1 individual [home range: 655 m; laboratory activity: 7]). This individual was excluded, and the deviation from expected growth rate was reanalyzed using the same linear model (data normally distributed [Shapiro–Wilks test: $P = 0.637$]).

RESULTS

General movements and frequency of detections

We recorded a decrease in the number of detected tags across the time, from 77% in the beginning to 58% at the end of the study. We detected 191 out of 192 individuals at least once during the study. The total number of recorded individual positions was 894. There was no relationship between laboratory activity and frequency of detections in the field (Spearman's $\rho = 0.093$; $P = 0.200$).

PIT-tag scanning in the stream revealed that most of the movements were occurring shortly after release. Thereafter movements largely ceased and positions were generally stable for individual

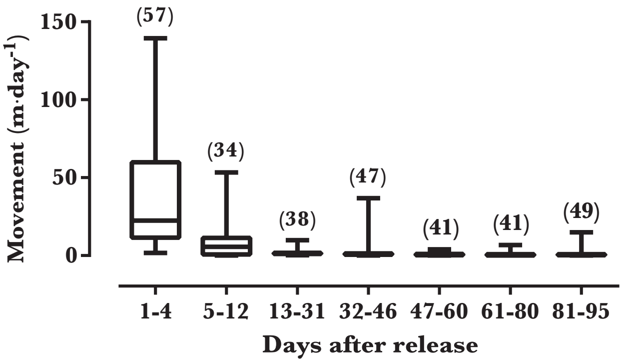

Figure 3

Mean movement per day of focal juvenile brown trout, which survived until the end of the experiment, between consecutive scannings. Boxplots (median with the box showing inter-quartile range) present the range of recorded movements, the number above each boxplot indicate sample size. Numbers on the *x* axis indicate a time span between the 2 consecutive scannings when the movements were recorded.

fish (Figure 3). Homing behavior of focal individuals was generally weak, and the majority (165 out of 192 focal individuals) did not attempt to migrate downstream the artificial step to the stream section where they were originally caught.

Lab activity and field movement strategy

Laboratory activity was positively related to initial dispersal in the wild ($F_{1,142}$ = 4.11; P = 0.044; Figure 4). There was no significant correlation between initial fish length and laboratory activity (Spearman´s ρ = 0.040, P = 0.578). However, initially larger fish moved longer distances in the wild ($F_{1,142}$ = 5.48; P = 0.021). Initial movement was not influenced by density treatment ($F_{1,142}$ = 0.29; P = 0.591). Neither was there any effect of the interaction between density and laboratory activity ($F_{1,141}$ = 0.64; P = 0.426).

Home range size was not associated with laboratory activity ($F_{1,70}$ = 0.65; P = 0.422) or initial size ($F_{1,70}$ = 0.51; P = 0.477).

Survival

There was no significant difference in laboratory activity among individuals determined as alive, dead, and nondetected at the last scanning of the experiment ($F_{2,189}$ = 1.48; P = 0.229): laboratory activity of alive (mean: 185; SE: 15.4; N = 73), dead (mean: 193; SE: 19.4; N = 32), and nondetected (mean: 158; SE: 13.1; N = 87) individuals.

Growth

There was no overall association between growth (i.e., deviation from expected specific growth rate) and laboratory activity ($F_{1,64}$ = 1.787; P = 0.186).

However, growth was significantly influenced by an interaction between laboratory activity and home range size ($F_{1,64}$ = 4.471; P = 0.038; Figure 5), where the growth of individuals with high laboratory activity decreased with increasing home range size, whereas the growth of individuals with lower laboratory activity increased slightly with increasing home range size.

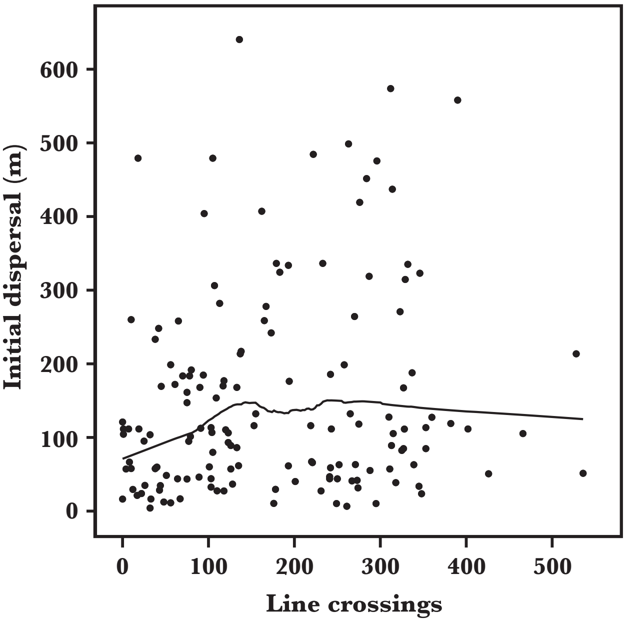

Figure 4
Correlation between laboratory activity (number of line crossings) and initial dispersal (4 days after release) of juvenile brown trout fitted by Loess line.

DISCUSSION

In this study, we investigated 1) to which degree laboratory activity is linked to movement strategies in the natural environment and 2) if individual differences in activity, growth, and survival in the wild are associated in concordance with the POLS hypothesis. We also aimed to investigate 3) how these associations were influenced by population density. However, because the initial dispersal of the fish was higher than expected and there were no significant effects of density, we will focus on the first 2 questions in the discussion below.

Association between activity in the lab and movement strategies in the stream

We found that laboratory activity was positively related to the initial dispersal of individuals. In contrast, home range size was not correlated with laboratory activity and all individuals were predominantly stationary after the initial dispersal. The positive relation between laboratory activity and initial dispersal provides evidence that open-field tests reflect ecologically significant behavioral traits of animal personality (i.e., activity and exploration, see Réale et al. 2007), which are linked to dispersal tendency (Dingemanse and de Goede 2004). Visual exploration of the Loess line plotted between the laboratory activity and distance of initial dispersal shows that the activity was positively related to initial dispersal only in individuals with low activity (up to a lab activity score of approximately 150). This can be an indication that a highly active fish, as scored in the laboratory, can be constrained in their movements under natural conditions, for example, by the stream environment (complexity, obstacles, open stream sections without refuges, high predation sites, etc.) An alternative explanation might be that the increased activity is associated with low predictability in a behavioral syndrome (Biro and Adriaenssens 2013).

Link between activity and life-history traits

Our second main question was whether laboratory activity in trout was associated with growth and survival in the wild and whether this association was consistent with the POLS hypothesis. The key assumption of this hypothesis, a positive correlation between activity, growth rate, and mortality (Biro et al. 2004, 2006; Stamps 2007; Cole and Quinn 2014), was not confirmed by our data, as there was no significant correlation between laboratory activity and growth rate or survival. Growth rate in more active individuals decreased with increasing home range size (i.e., decrease of food abundance), whereas the growth of individuals with lower activity increased slightly with increasing home range size, indicating an interaction between life-history traits, environmental conditions, and behavioral traits linked to animal personality. Assuming a complete concordance (i.e., negative correlation) between food abundance and home range size is an oversimplification of the natural system because home range size in salmonids is determined by multiple interacting factors including predation risk (Kim et al. 2011), competition (Keeley 2000), and habitat complexity (Kalleberg 1958). However, the energetic trade-off selects for the smallest home range size, which satisfies the energetic demands of the individual. Thus, an increase in food abundance is generally assumed to reduce the home range size (Hixon 1981; Grant 1997; Slavík et al. 2014).

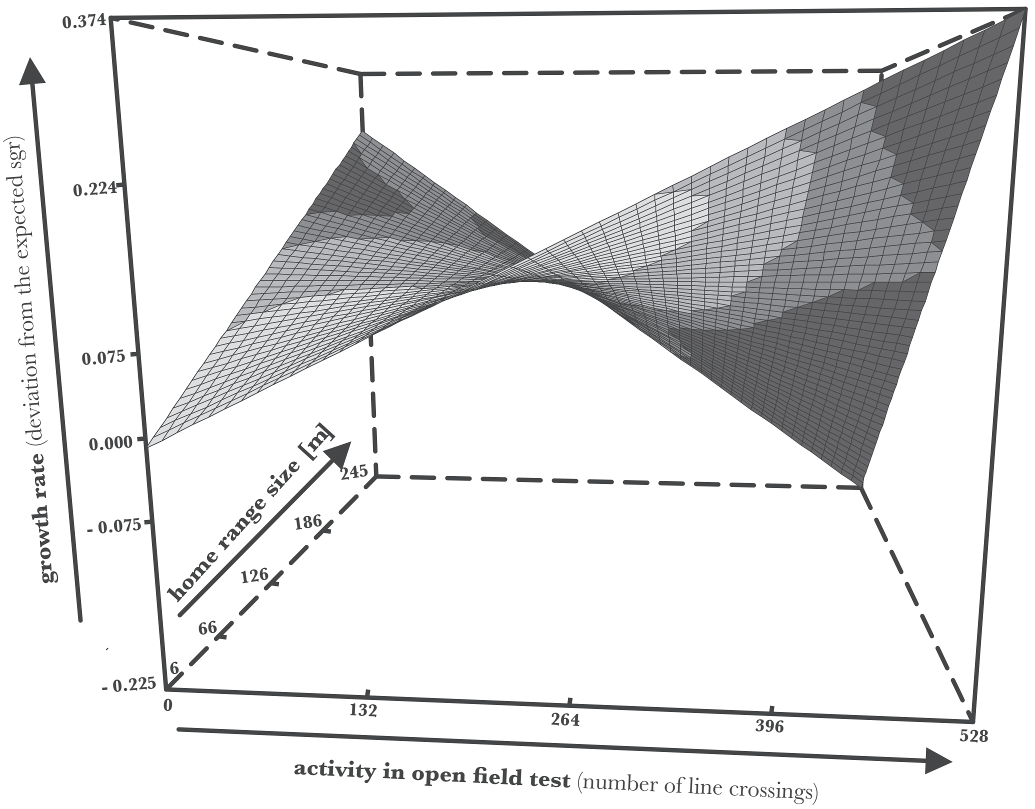

Figure 5
Surface plot displaying effect of interaction between laboratory activity and home range size on growth rate (i.e., deviation from the expected growth rate) in juvenile brown trout. The highest 75%, 50%, and 25% of the bivariate density estimate of laboratory activity and home range size are colored in with colors of increasing gray scale.

We propose that more active individuals adopt a high gain/high cost strategy, as described in Metcalfe (1986), and are therefore able to yield a high net energy income and growth rate when conditions are optimal (e.g., high food availability). However, they suffer by low net energy income and growth rate under suboptimal conditions (low food availability) due to high activity–related maintenance costs. In contrast, less active individuals adopt a low gain/low cost strategy, allowing them to maintain a more constant growth rate under a wider range of environmental conditions (Figure 6). Thus, the link between these alternative strategies and fitness (i.e., growth) is dependent on environmental conditions. Although activity certainly increases metabolism, other characteristics of individual affecting energetic costs, like resting metabolic rate, are often (Biro and Stamps 2010; Réale et al. 2010), but not always (Houston 2010) positively correlated with activity.

Implications for POLS hypothesis: a new conceptual model

We suggest along with previous studies (Dingemanse et al. 2004; Dingemanse and de Goede 2004; Adriaenssens and Johnsson 2009, 2011, 2013; Höjesjö et al. 2011; Le Galliard et al. 2013; Montiglio et al. 2014) that the POLS hypothesis in its original form is too simplistic to explain the adaptive value of consistent behavioral traits under natural conditions, as exemplified by our stream model system, a complex and unpredictable environment with fluctuating selective pressures (Höjesjö et al. 2004). Based on the results of our field experiment and previous studies in similar experimental systems, we suggest an alternative hypothesis, complementary to the POLS concept (Réale et al. 2010), linking consistent interindividual variation in activity to reaction norms associating food abundance and growth rate (Figure 6). The general validity of our conceptual model is tentatively supported by other empirical studies conducted in similar experimental systems (Biro et al. 2004, 2006; Adriaenssens and Johnsson 2011, 2013; Höjesjö et al. 2011). When summarized, the results of these studies are consistent with the hypothesis that different reaction norms link food abundance and growth rate for alternative behavioral strategies (Figure 6): in environments with stable and rich food habitats, more active individuals grow faster than passive because they are able to utilize abundant and predictable food resources (Biro et al. 2004, 2006), whereas environments with less predictable food abundance do not always meet costs of high activity and therefore passive or shy individuals can grow as fast as, or even faster than, active or bold individuals (Adriaenssens and Johnsson 2011, 2013; Höjesjö et al. 2011). The ecological relevance of these reaction norms may be even more general, also including other environmental variables where growth performance is optimal at intermediate conditions. For example, Armstrong et al. (2011) hypothesized similar curves for describing the relationship between growth and water velocity in salmon (*Salmo salar*) with different standard metabolic rate (gray lines in Figure 6). Even more generally, these norms could be viewed as an analogy to the performance of generalist and specialist strategies over a range of environmental conditions (Gilchrist 1995).

Frequency-dependent selection acting in a fluctuating environment may allow coexistence of different activity in a natural population (Maynard Smith 1982). For example, according to our hypothesis (Figure 6), a population of mostly passive individuals

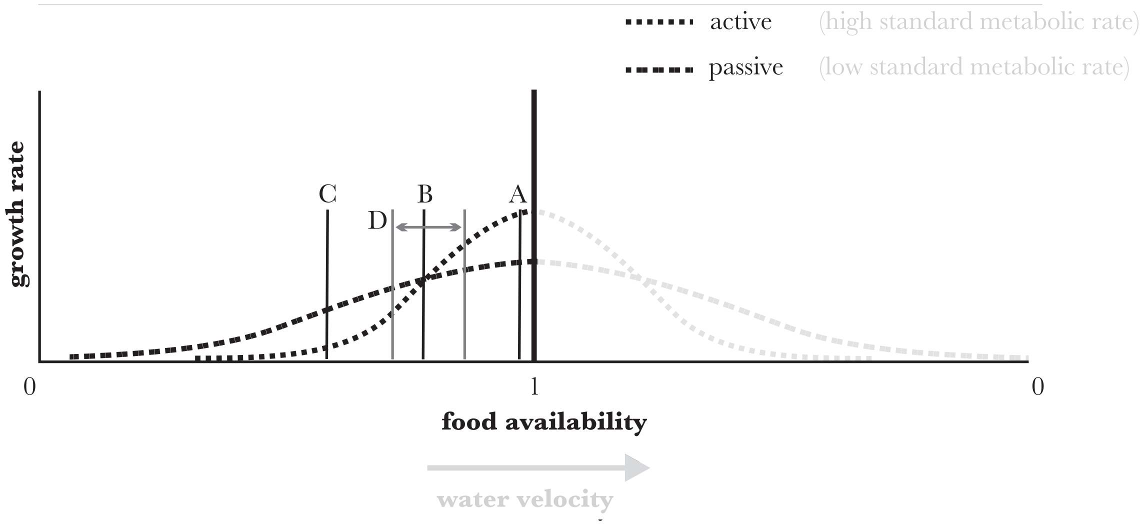

Figure 6
The hypothetical reaction norm associating environmental variation to growth for individuals adopting an extreme active or passive strategy, respectively. The units are arbitrary. Food availability is increasing from 0 to 1 (1 is equal to optimal conditions when individuals can feed ad lib). Letters denote studies evaluating the growth of freshwater salmonid fish with different levels of activity in environments assumed to differ in food abundance—A: Biro et al. (2004, 2006) (conducted on wild and domesticated rainbow trout *Oncorhynchus mykiss*); B: Adriaenssens and Johnsson (2013) (conducted on wild brown trout) and Höjesjö et al. (2011) (hatchery reared wild brown trout); C: Adriaenssens and Johnsson (2011) (conducted on wild brown trout); D: present study (conducted on wild brown trout)—interval of values between dark gray vertical lines. Note that in the studies Biro et al. (2004, 2006), boldness was used as an explanatory behavioral trait; however, bold individuals in these studies were also more active. Shaded parts represent extended hypothetical reaction norms for variables where performance is assumed to be optimal at intermediate conditions (i.e., water velocity with a high level of food availability—Armstrong et al. 2011). Water velocity increases from the left to right.

can be invaded by a high-activity mutant at optimal or near optimal environmental conditions, but the fitness of the mutant strategy will vary more in response to environmental changes, allowing the passive strategy to increase in response to unpredictable fluctuating environmental conditions. Maternal effect has been shown to drive behavior and growth in juvenile brown trout (Höjesjö et al. 2011). Therefore, a parental bet-hedging strategy producing offspring with mixed activity can maintain within-family variation of behavior in unpredictable natural environments (Hamer et al. 2002).

CONCLUSIONS

In summary, we showed that laboratory activity often measured as behavioral traits in animal personality research (i.e., activity and exploration; Réale et al. 2007) associated with initial dispersal and growth of juvenile brown trout in the wild. However, our results do not fully support the original version of the pace-of-life-syndrome hypothesis as we found no constant trade-off between laboratory activity, growth rate, and survival. Taken together with previous studies (Biro et al. 2004, 2006; Adriaenssens and Johnsson 2011, 2013; Armstrong et al. 2011; Höjesjö et al. 2011; Figure 6), our results are more consistent with the hypothesis that the growth of individuals adopting active behavioral strategies is more sensitive to deviations from optimal environmental conditions than the growth of individuals adopting passive strategies.

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PAPER V

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Validation of scale-reading estimates of age and growth in a brown trout *Salmo trutta* population

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Abstract: In this study, we validated a scale-reading method estimating age and growth in brown trout *Salmo trutta* in wild, landlocked, stream-dwelling populations from mountain headwaters in the Elbe catchment area of the Czech Republic. The values estimated from scale reading were compared with measured values, collected using a mark-recapture program over eight consecutive years. The age-corrected absolute percentage error was 10.71%, primarily because the ages of the oldest individuals according to scale reading were underestimated, and the ages of juvenile individuals were slightly overestimated. The back-calculated length was slightly underestimated (the mean error was −4.60 mm), but it was not significantly different from the real measured length. This study suggests that in cold mountain headwaters, scale reading is a sufficiently accurate method for age and growth estimation in juvenile brown trout; however, the results for adult individuals must be taken with caution.

Key words: ageing; growth back-calculation; mark-recapture; fish

Introduction

Fishery scientists and managers measure individual growth and age structure to describe fish population dynamics and evaluate fish stock management (Leonardos 2001; Yule et al. 2008). The age and growth estimates from calcified bony structures are commonly used for many ectothermic species, such as fish (Das 1994), amphibians (Liao 2011) or reptiles (Castanet 1994). Scales, otoliths, spines, vertebrae, fin rays and skull bones are typically used for age and growth estimates in fish (Helfman et al. 2009).

Estimating age and back-calculating growth through scale reading is based on annuli counts along the anterior-posterior axis of a scale (Lee 1920; Pearson 1928) and it is the oldest, but still commonly used method (Alvord 1954; Maceina et al. 2007). The primary advantage of scale reading is that sampling is nonlethal for fish and has no influence on viability, whereas removing bony structures such as otoliths requires sacrificing the fish (Hubert et al. 1987). Therefore, scale reading is especially useful if the target population is endangered or protected because it facilitates harmless evaluation of many individuals in a population. However, lower precision in age and growth estimates from scales compared with other calcified structures has been reported, for example in van der Meulen et al. (2013).

Scales grow by accretion at their margins and form annuli during a regular period of annual slow growth (Helfman et al. 2009), which is determined by low water temperatures during winter seasons in temperate European climates (Elliott 1989). Variability in individual growth across seasons is caused by many endogenous and exogenous stress factors, such as disease, injury, food and nutrient unavailability, maturation, reproductive behaviour and temperature (DeVries & Frie 1996; Helfman et al. 2009; Zhi-Hua et al. 2010). Such growth variability can be associated with irregularity in annuli formation on scales, which can lead to errors in age and growth estimates (Beamish & McFarlane 1983). Age and growth estimates in fish populations based on scale reading are likely less precise for populations with long and indistinct growing seasons (Hoxmeier et al. 2001). On the other hand, slow growth may produce crowded annuli, which decrease the precision of scale reading, and as a result, diminish the validity of this information (Power 1978). Thus, knowledge of scale reading precision is necessary to validate such information (Beamish & McFarlane 1983; Horká et al. 2010; Lopez Cazorla & Sidorkewicj 2011).

The aim of this study was to validate age and growth estimates from scale reading in a landlocked population of brown trout *Salmo trutta* L., 1758, which is a widespread and ecologically important species of

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European salmonids (Kottelat 1997). The estimates from scale reading were compared with results obtained from measuring values through mark-recapture observations in mountain headwaters in Czech Republic.

Material and methods

In total, 6,240 of brown trout individuals were caught in the headwaters of the Otava River (49°1′ N, 13°29′ E) and the Teplá Vltava River (48°58′ N, 13°39′ E) in the Elbe catchment area, the Czech Republic. Both sampled streams had similar hydrological conditions and species structure. For a detailed description of the study site and fish population, see Slavík et al. (2012) and Závorka et al. (2013). The fish were sampled twice a year (May and October) from autumn 2005 to autumn 2012 and were caught with a backpack electro-fishing device (EFKO, Germany). Every specimen was anesthetized (2-phenoxiethanol), measured (standard length to the nearest mm), weighed (to the nearest g), and individually tagged using Visible Implant Alphanumeric tags (Northwest Marine Technology, Shaw Island, Washington) or passive integrated transponder (PIT) tags (Trovan, UK). From 1,754 randomly selected individuals, 5–10 scales were sampled. The scales were removed from the left body side, one row above the lateral line in the intersection of dorsal and ventral fin (DeVries & Frie 1996).

The scales were placed between two microscope slides, magnified (67.5 times), and photographed with a camera connected to a stereoscopic microscope (Arsenal, www.arsenal.cz, Czech Republic). The scales from 7% of the fish sampled were discarded because they were distorted or scarred. The scale readings were conducted without prior knowledge of the fish size. The age was determined using a standard notation following Pearson (1928), which involved counting winter annuli. The scale size and annual increments were measured along the anterior-posterior line from the scale centre to its margin (Martinson et al. 2000) using picture analysis software (UTHSCSA ImageTool 3.0., http://compdent.uthscsa.edu/dig/itdesc.html, USA). Growth was back-calculated using the Fraser-Lee equation (Lee 1920), which is defined as follows:

$$L_t = \frac{S_t}{S_c}(L_c - c) + c \tag{1}$$

where L_t is the length at age t, L_c is the total length, S_t is the radius of a scale annulus at age t, S_c is the scale radius, and c is the empirical constant. The value of constant c was 10, estimated based on the brown trout larval length in mm (Baruš & Oliva 1995; Ojanguren & Braña 2003). In the case of this study, the calculation of the constant c as the intercept from the length-scale relationship regression was inappropriate, as it yielded unrealistic values for the back-calculated length.

The scale reading validation was based on 53 recaptured individuals, for whom both initial and recapture scale samples were available. The recaptured individuals were exposed in the stream for seven months in average (ranging from 4 to 25 months). Seventeen individuals were exposed to the winter conditions, while 36 individuals were exposed in stream from spring to autumn between the capture. The individuals' age at recapture was compared with the "standard age", which was calculated as the initial capture age plus the time between captures. We used the earlier estimate as a reference for the "standard age", because it is generally accepted that age estimates in younger fish are more precise (DeVries & Frie 1996). Two coefficients were used to express the error in aging. The standard percentage error, which is the ratio between the number of errors in aging and the sample size, and the absolute percentage error (PE) relative to standard age (equation 2) were used. The absolute percentage error measures the error size in relation to the fish age (Rifflart et al. 2006), as follows:

$$\mathrm{PE} = 100\frac{1}{N}\sum_{i=1}^{N}\left(\frac{|X_i - Y_i|}{X_i}\right) \tag{2}$$

where N is the sample size, X_i is the standard age of the i^{th} fish and Y_i is the estimated age of the i^{th} fish. The precision of the initial capture age estimates was evaluated by two independent skilled readers. The error of estimation was calculated using the following coefficient of variation by Chang (1982):

$$\mathrm{CV} = 100\frac{1}{N}\sum_{j=1}^{N}\frac{\sqrt{\sum_{i=1}^{R}\frac{(X_{ij} - X_j)^2}{R-1}}}{X_j} \tag{3}$$

where N is the sample size, R is the number of times each fish age was determined, X_{ij} is the i^{th} age determination for the j^{th} fish, and X_j is the average age calculated for the j^{th} fish.

The back-calculated length accuracy was assessed using a Virtual Mark procedure, in which the structure radius at the initial capture was modelled on the recaptured sample (Zymonas & McMahon 2009) as follows:

$$S_{\mathrm{VM}} = A_{n\mathrm{R}}\left(\frac{S_{\mathrm{CI}}}{A_{n\mathrm{I}}}\right) \tag{4a}$$

$$L_{\mathrm{VM}} = \left[\frac{(L_{\mathrm{R}} - c)}{S_{\mathrm{CR}}}\right]S_{\mathrm{VM}} + c \tag{4b}$$

where S_{VM} is the scale radius at the virtual mark, S_{CI} is the scale radius at the initial capture, S_{CR} is the scale radius at recapture, $A_{n\mathrm{R}}$ is the n^{th} annulus radius at recapture, $A_{n\mathrm{I}}$ is the n^{th} annulus radius at the initial capture, L_{VM} is the back-calculated fish length at the virtual mark, L_{R} is the fish length at recapture, and c is the empirical constant. The accuracy of the back-calculation was validated only in individuals with correctly estimated age in both samples (initial capture and recapture). The differences between the back-calculated and measured lengths were evaluated using a paired t-test.

Results

The estimated age of captured fish ranged from 1+ to 8+. The age of the recaptured fish was estimated with an overall 20.80% standard error and a 10.71% absolute age-corrected percentage error ($n = 53$). The lower standard error and the lower absolute percentage error in younger age groups were found (Table 1). The scale reading accuracy was affected neither by winter conditions, nor the exposition time (Table 2). The variation coefficient of the age estimates in two independent readers was 7.46 ($n = 53$). The accordance of reader A

Table 1. Error rate (%) in age estimation according to the time of exposition; 95% confidence interval is given for standard error percentage only.

Number of winters (range of exposition time in days)	Number of fish	Error					
		Number	Overestimated	Underestimated	Standard error percentage	Confidence interval (95%)	Age corrected error percentage
Zero (124–161)	36	8	4	4	22.2%	8.0–36.5	10.7%
One (211–365)	14	1	0	1	7.1%	0.0–22.6	2.4%
More (571–720)	3	2	0	2	66.7%	0.0–100.0	25.8%
Total	53	11	4	7	20.8%	9.5–32.0	10.7%

Table 2. Error rate (%) in age estimation according to age classes; 95% confidence interval is given for standard error percentage only.

Age	Number of fish	Error			Standard error percentage	Confidence interval (95%)	Age corrected error percentage
		Number	Overestimated	Underestimated			
1+	12	1	1	0	8.3%	0.0–26.7	8.3%
2+	30	5	5	0	16.7%	2.5–30.8	10%
3+	7	2	1	1	28.6%	16.6–73.7	9.5%
4+	2	1	0	1	50%	0.0–100	12.5%
5+	1	1	0	1	100%	–	40%
6+	0	–	–	–	–	–	–
7+	0	–	–	–	–	–	–
8+	1	1	0	1	100%	–	37.5%
Total	53	11	7	4	20.8%	9.5–32.0	10.7%

Table 3. Age frequency table summarizing pairwise comparison of age estimates from two readers. Data indicate the number of fish individuals.

		Reader B								
	Age	1+	2+	3+	4+	5+	6+	7+	8+	Total
Reader A	1+	10	2							12
	2+	2	21	7						30
	3+		2	4	1					7
	4+			2	0					2
	5+			1		0				1
	6+									0
	7+									0
	8+						1		0	1

and reader B decreased with increasing estimated age of fish, being highest in the age classes 1+ and 2+ (Table 3).

The size of the individuals tested ranged from 73 mm to 276 mm at the initial capture, and 115 mm to 290 mm at the recapture. The measured length did not differ from the back-calculated length for the Virtual Mark (paired t-test: $t = -0.91$, df $= 76$, $P = 0.37$). The mean for the differences was –4.60 mm (see Fig. 1).

Discussion

Our results showed higher errors in age and growth estimates compared to studies using otoliths (Hubert et al. 1987; Schill et al. 2010); the age and length were slightly underestimated, which is consistent with results from earlier studies focused on scale-reading validation (Kruse et al. 1997; Rifflart et al. 2006; Heidarsson et al. 2006; Zymonas & McMahon 2009). We found a tendency to overestimate the age of juvenile individuals; however, the results indicated generally bigger errors in adult individuals. This assumption was also supported by the finding that the accordance of two independent readers decreased with increasing age of individuals. We suggest that the energetic requirements for breeding together with the harsh environmental conditions in mountain headwaters decrease the growth of mature trouts (Bohlin et al. 2001) and may lead to annuli crowding. Consequently, the crowded annuli increased the risk of age underestimation in mature individuals (Alvord 1954; Power 1978). The time of exposition in stream and number of winters between captures, which could increase the risk of erroneous scale reading, did not affect the precision of scale reading.

To summarize our results, the age and growth estimates based on scale reading were found to be a suffi-

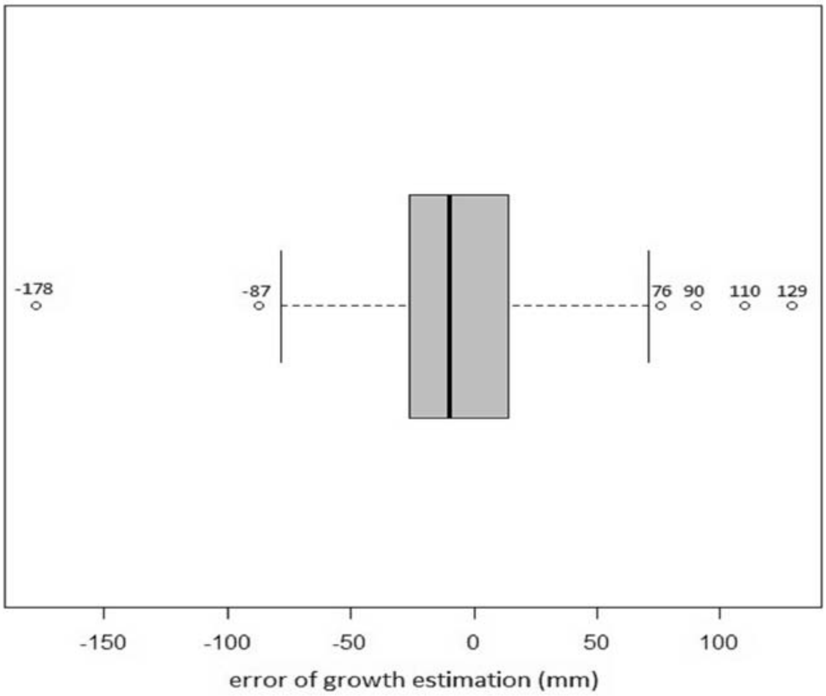

Fig. 1. Boxplot of error distribution in length back-calculation. Errors are expressed as a difference between the real measured length and the length modelled based on the Virtual Mark procedure (equations 4a and 4b). Outlying values are marked with circles accompanied with their values in mm.

ciently precise method for juvenile individuals until maturity, which included the fish in age classes 1+ and 2+ for the observed streams (Baruš & Oliva, 1995). However, scale-reading does not provide reliable estimates of age and growth for adult individuals in brown trout dwellings in cold mountain streams. These findings seem to be of importance for field biologists and managers focused on populations of free living salmonids in cold waters.

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PAPER VI

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Distribution and growth of brown trout in pristine headwaters of Central Europe

Research Article

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Abstract: The majority of stream-dwelling salmonid populations in Europe are affected by artificial stocking and the fragmentation of riverine ecosystems. The present study was performed in the unique pristine headwaters of the Otava River in the Elbe catchment area of the Czech Republic. The aim was to investigate the spatial distribution and individual growth pattern of brown trout, *Salmo trutta*, populations. Twenty sites in two main streams and their tributaries were sampled twice a year (spring and autumn) during the period 2005–2011. The sampling sites were grouped into fourteen so-called synchronised population units within the boundaries of three populations, according to analyses of synchrony in population abundance. The individual growth of juveniles (age-0, age-1) varied between all three spatial units (sampling sites, synchronised population units and populations), while the individual growth of adults (age-2 and older) did not. The distinctiveness regarding individual growth and demographic independence among the synchronised population units and populations indicates their suitability for use as population units for management purposes.

Keywords: *Demographic synchrony • Salmonid fish • Body length increment • Ontogeny • Metapopulation*

1. Introduction

In poikilothermic organisms, body size, as a direct consequence of individual growth, is strongly correlated with many physiological traits [1]. The key role of individual growth as a driver of population dynamics in stream-dwelling salmonids has been widely reported [2-6]. Individual growth influences brown trout, *Salmo trutta*, populations beginning in the early ontogenetic phases, as demonstrated by the positive correlation between juvenile survival and body size [7]. However, this relationship is highly variable across seasons and populations [8]. In the later phases of the life cycle, individual growth affects the fertility of individuals, as the number and weight of eggs increase with female body size [2], and individuals that grow faster achieve earlier sexual maturation than their conspecifics [9]. Throughout their life span, the growth of trout has an influence on their competitive ability, which increases with body size [10], and migration behaviour, as larger and faster growing individuals show higher mobility [11,12]. Generally, growth has an essential influence on the fitness of individuals [13], and variations in growth trajectories can have a substantial effect on brown trout population dynamics [5].

Stream-dwelling salmonids often exhibit a high level of population differentiation [14,15]. Partially isolated brown trout populations have been observed at local geographical scale in streams fragmented by migration barriers [16,17] as well as in streams with free migration corridors, where the populations has been isolated by distance [18,19]. Isolation among populations or among

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smaller population units can lead to differentiation in life traits, such as individual growth [11,20].

The aim of the present study was to investigate spatial distribution and the individual growth patterns of brown trout populations in the headwaters of the Otava River. To our knowledge, this is the first intensive study addressing individual growth and the spatial population distribution in brown trout in headwaters within central Europe.

2. Experimental Procedures

2.1 Study area

The headwaters of the Otava River are located in Šumava National Park in the Czech Republic (49°1'N, 13°29'E; Figure 1). The relief of the landscape in the Šumava National Park region is mountainous, and the most widespread vegetation type is spruce forest, which alternates with meadows and peat bogs. The studied headwater streams consist of two main tributaries, the Vydra and Křemelná Rivers, which spring at 1,100 m a.s.l. and achieve confluence after *ca*. 30 km, creating the Otava River. The overall area of the Vydra and Křemelná basins is approximately 224 km^2. The study streams are oligotrophic and pristine conditions prevail. Twenty sampling sites were chosen along the longitudinal gradients of the study streams and their tributaries (Table 1) according to National Park access permission. Nine of the sampling sites had a riparian canopy, while nine flowed through meadows and peat bogs. The average flow at the sampling sites ranged from 0.01 to 2 m^3 s^{-1}. The substratum of stream beds was heterogeneous and contained sand, gravel, pebbles and boulders (Table 1). No obstacles prevent migration; the only exception was found in Švelský Stream, where there is a natural, impassable 2.5 m-high steep boulder located approximately 80 m from confluence with the Vydra River. The boulder barrier prevented upstream migration to this tributary.

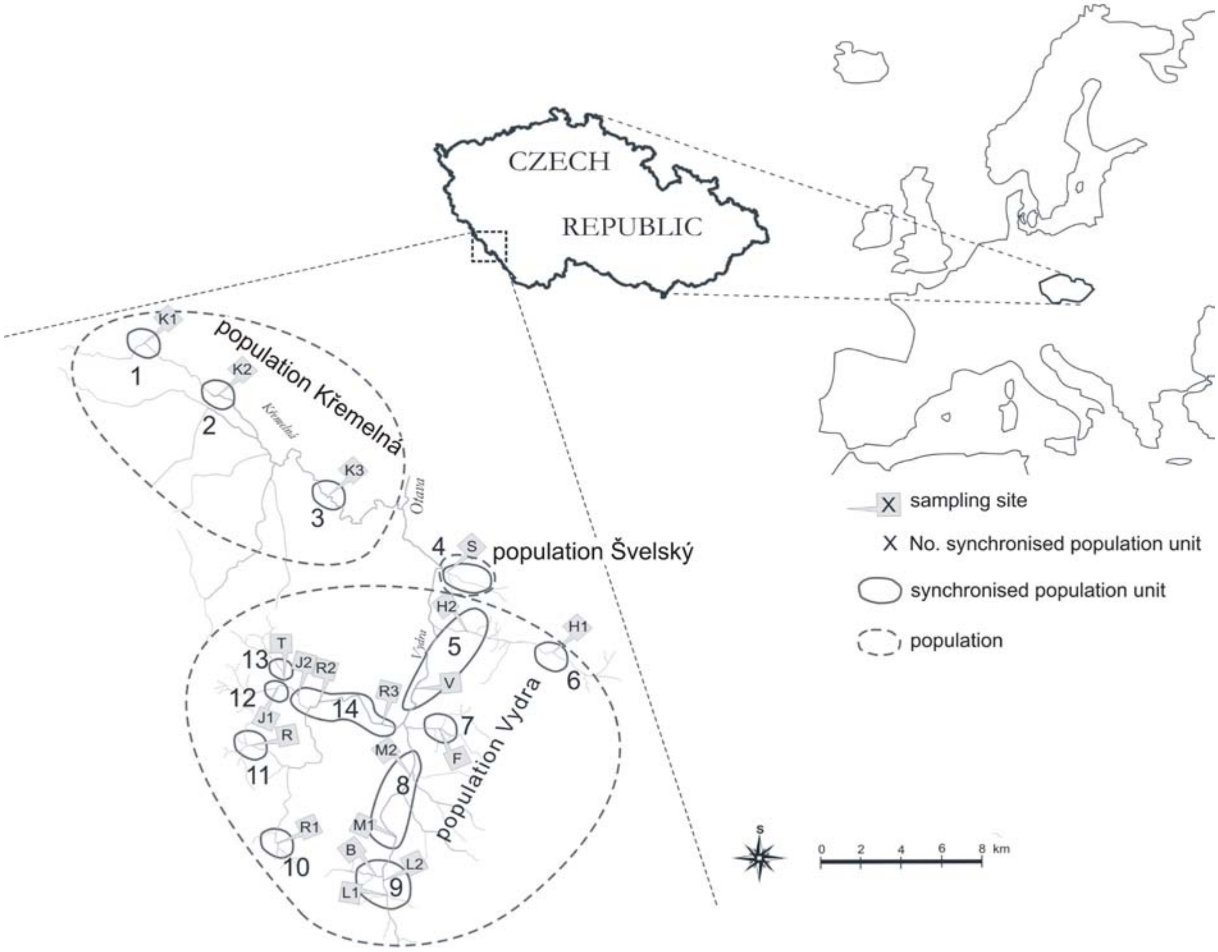

Figure 1. Map showing locations of study area with highlighted sampling sites, synchronized population units and populations.

Sampling site	Sampling site ID	Synchnize population unit	Population	Temperature (°C)	pH	Slope (‰)	Dominant substrate (%)	Substratum size (mm)
Křemelná_1	K1	1	Křemelná	7.4 (4.6 - 14.0)	6.3 (5.5 - 7.7)	0.9	gravel (64)	72 (1 - 350)
Křemelná_2	K2	2	Křemelná	9.6 (6.2 - 17.0)	6.7 (5.6 - 8.1)	1.24	gravel (45)	110 (1 - 650)
Křemelná_3	K3	3	Křemelná	10.1 (7.0 - 13.7)	6.5 (4.5 - 7.7)	1.6	gravel (48)	145 (1 - 825)
Švelský	S	4	Švelský	7.1 (3.5 - 9.0)	5.8 (4.2 - 6.7)	35	boulders (51)	314 (1 - 1580)
Hamerský_2	H2	5	Vydra	8.5 (4.4 - 12.6)	6.3 (4.2 - 7.7)	9.56	pebbles (38)	260 (1 - 1030)
Vydra	V	5	Vydra	7.1 (4.5 - 14.1)	5.8 (4.5 - 7.1)	2.45	pebbles (53)	278 (2 - 1370)
Hamerský_1	H1	6	Vydra	7.8 (4.1 - 10.3)	5.6 (4.2 - 7.8)	2.34	gravel (57)	72 (1 - 410)
Filipohuťský	F	7	Vydra	7.4 (3.1 - 9.9)	5.3. (3.8 - 7.8)	3.56	pebbles (48)	111 (1 - 610)
Modravský_1	M1	8	Vydra	9.1 (4.2 - 14.5)	6.4 (5.4 - 7.3)	2.78	pebbles (57)	263 (2 - 860)
Modravský_2	M2	8	Vydra	9.1 (7.1 - 14.5)	6.2 (5.3 - 7.3)	2.43	boulders (42)	294 (2 - 1200)
Březnický	B	9	Vydra	8.4 (3.4 - 12.4)	5.6 (3.8 - 7.1)	6.13	pebbles (59)	147 (1 - 880)
Luzenský_1	L1	9	Vydra	8.2 (4.7 - 11.2)	5.5 (4.2 - 7.7)	1.56	gravel (75)	103 (2 - 650)
Luzenský_2	L2	9	Vydra	8.8 (6.4 - 12.2)	5 (3.4 - 7.3)	0.89	gravel (51)	122 (1 - 850)
Roklanský_1	R1	10	Vydra	8.0 (6.1 - 10.0)	5.5 (4.0 - 7.2)	2.27	gravel (83)	61 (1 - 350)
Rokytka	R	11	Vydra	6.2 (2.3 - 9.7)	5.6 (3.5 - 7.4)	0.68	gravel (83)	61 (1 - 350)
Javoří_1	J1	12	Vydra	6.5 (2.9 - 9.4)	5.8 (4.1 - 8.0)	1.72	pebbles (64)	138 (5 - 450)
Tmavý	T	13	Vydra	6.6 (3.1 - 10.5)	5.6 (3.6 - 7.3)	3.78	pebbles (57)	101 (10 - 285)
Roklanský_3	R3	14	Vydra	8.8 (4.0 - 14.0)	6.0 (4.6 - 7.3)	1.6	pebbles (52)	181 (1 - 980)
Javoří_2	J2	14	Vydra	7.7 (3.5 - 11.4)	6.3 (4.1 - 8.8)	1.87	pebbles (52)	181 (1 - 980)
Roklanský_2	R2	14	Vydra	8.8 (3.5 - 13.4)	6.2 (4.6 - 7.4)	1.87	pebbles (52)	181 (1 - 980)

Table 1. Spatial structure of brown trout population within studied sites and variability of selected abiotic factors (means, range in brackets provided).

Fishing is banned and no stocking occurs in study streams. Therefore, the local ichthyofauna includes populations of autochthonous species. Only brown trout occurs in study streams and is accompanied by bullhead *Cottus gobio* in Křemelná River.

2.2 Data collection and analyses

Sampling of fish was performed at 20 sampling sites twice a year (in May and October) during seven consecutive years, from autumn 2005 to autumn 2011. Fish were captured using a backpack electro shocker (EFKO, Germany). A single pass electrofishing method was used, which is considered sufficient for the determination of brown trout abundance in mountain headwater streams [21,22]. The location and assessed area of sampling sites as well as the fishing effort were maintained constant throughout the study period. Every specimen was measured (standard length to the nearest mm), weighed (to the nearest g) and individually tagged at the lower left jaw using VIA (visible implant alphanumeric) tags (Northwest Marine Technology, USA). Specimens that were of insufficient size for individual tagging (standard length smaller than 90 mm) were marked using VIE (visible implant elastomer) tags (Northwest Marine Technology, USA). The detection of tagged fish was noticed as recapture. Scale samples were obtained from 709 randomly selected individuals.

The morphological parameters of the sampling sites were measured once, at the beginning of the study. The river slope (%) was measured using a Pulse Total Station (Topcon GPT 2000, Itabashi, Tokyo, Japan) and was determined for the stretches delineated by fish sampling. The river slope was considered to correspond to the difference between water levels in two adjacent stream cross-sections [23]. The river substratum was quantified according to Wolman [24]. Water temperature and pH (WTW, pH/Cond 340i SET) were measured before every sampling event (Table 1).

The individual growth and age of fish were estimated *via* scale readings performed along the anterior-posterior axis of scales [25]. For these readings, only fully developed scales were used, and regenerated or distorted scales were disregarded. Age was estimated by examining winter annuli, and growth was back-calculated using the Fraser-Lea formula [26]. Because of the well-known high level of estimation errors that occur when ageing salmonids after the third year of life [27,28], individuals older than three years were grouped in a single category for all analyses.

The data obtained from the mark-recapture program were used in analyses of recapture rate and movements. The recapture rate was calculated as the percentage of recaptured individuals among the total number of marked individuals. Site fidelity was measured as the percentage of recaptured individuals at their original tagging site among the total number of recaptured individuals. Dispersal distance was calculated as the average distance travelled by recaptured individuals among sampling sites.

The twenty sampling sites were linked together into demographically independent population units. This grouping was assessed *via* synchronisation of demographic dynamics among sampling sites, considering the existence of migration obstacles and individual migration behaviour. To examine the demographic synchrony among sampling sites, we determined the Pearson moment correlation of seasonal (spring, autumn) growth rates of individual's abundance between pairs of sampling sites [29]. The growth rate was expressed as the change in a number of individuals over yearly increments, expressed as the percentage of year-1 values (see Petranka *et al.* [30]). Sampling sites in which at least one zero abundance result occurred during the total period of observation were excluded from the analyses (there were four excluded sampling sites in spring and three in autumn). The spatial extent of synchrony among sampling sites was estimated as the x-intercept of the linear regression of the correlation coefficient of individual's abundance growth rate on sampling site distance [29,31]. The analyses were performed separately for each season. Sampling sites were grouped into populations based on the spatial synchrony of individual's abundance growth rate and the occurrence of impassable migration obstacles. Sampling sites within single populations were further sorted into synchronised population units. Sampling sites with a mean correlation coefficient of demographic synchrony that was higher than moderate (ρ=0.56; see Koizumi *et al.* [32]) and with a distance between them smaller than the average dispersal distance of individuals in the focal area [30] were grouped into a synchronised population unit. Sampling sites that were not included in the analyses of demographic synchrony were grouped with the nearest sampling site if the distance between them was smaller than the average dispersal distance of individuals in the focal area. Otherwise, they were considered independent synchronised population units.

Associations between the variables related to the individual growth variation were tested using a linear mixed model (LMM). The data were transformed for normality prior to LMM analyses when necessary. To account for repeated measures, all analyses were performed using a mixed model with random factors (PROC MIXED; SAS, Version 9.1; SAS Institute Inc.; www.sas.com). Separate models were applied for the following dependent variables: *brown trout abundance throughout season* (LMM I; fixed factor: season; random factors: locality, year and their mutual interaction); *individual growth throughout ontogenesis* (hereafter assessed on the basis of scale readings; LMM II; fixed factor: age; random factors: locality, year, scale samples and their mutual interactions); *individual growth of age-0 across the sampling site spatial units* (LMM III; fixed factor: sampling site; random factors: year, scale samples and their interaction); *individual growth of age-0 across the synchronised spatial population units* (LMM IV; fixed factor: synchronised population unit; random factors: year, scale samples and their mutual interaction); *individual growth of age-0 across the population spatial units* (LMM V; fixed factor: population; random factors: year, scale samples and their mutual interaction); *individual growth of age-1 across the sampling site spatial units* (LMM VI; fixed factor: sampling site; random factors: year, scale samples and their mutual interaction); *individual growth of age-1 across the synchronised population spatial units* (LMM VII; fixed factor: synchronised population unit; random factors: year, scale samples and their mutual interaction); *individual growth of age-1 across the population spatial units* (LMM VIII; fixed factor: population; random factors: year, scale samples and their mutual interaction); *individual growth of age-2 across the sampling site spatial units* (LMM IX; fixed factor: sampling site; random factors: year, scale samples and their mutual interaction); *individual growth of age-2 across the synchronised population spatial units* (LMM X; fixed factor: synchronised population unit; random factors: year, scale samples and their mutual interaction); *individual growth of age-2 across the population spatial units* (LMM XI; fixed factor: population; random factors: year, scale samples and their mutual interaction); *individual growth of age-3 and older across* *the sampling site spatial units* (LMM XII; fixed factor: sampling site; random factors: year, scale samples and their mutual interaction); *individual growth of age-3 and older across the synchronised population spatial units* (LMM XIII; fixed factor: synchronised population unit; random factors: year, scale samples and their mutual interaction); *individual growth of age-3 and older across the population spatial units* (LMM XIV; fixed factor: population; random factors: year, scale samples and their mutual interaction). The significance of each fixed effect in the mixed LMM models was assessed using an F-test. Least-squares means (LSM), henceforth referred to as adjusted means, were computed for each class, and differences between classes were tested using a t-test. For multiple comparisons, we used a Tukey-Kramer adjustment. The degrees of freedom were calculated using the Kenward-Roger method [33].

3. Results

3.1 Mark-recapture analyses

A total of 5195 individual brown trout were caught and tagged throughout the study period. Total abundance differed across seasons, being higher during autumn (LMM I; $F_{1;216}$=139.47, P<0.0001; Adj. P<0.0001). The overall recapture rate was 9%. The recaptured individuals were largely caught only once (89%), though some were caught twice (10%) or three times (1%). The majority of recaptured individuals (92%) displayed site fidelity. Those that were caught outside of their original tagging site were predominantly found in adjacent ones. The average distance of recaptured individuals travelling among sampling sites was 5828 m.

3.2 Demographic synchrony and population distribution

The linear regression analysis of demographic synchrony among sampling sites was significant only for autumn season. The spring season was therefore excluded from further analyses. The spatial extent of synchrony among sampling sites in autumn was estimated as the x-intercept of the linear regression of the correlation coefficient of individuals abundance growth rate on sampling site distance (r=-0.39, P>0.0001, n=120; y=-14.56x+17938.47). According to the extent of demographic synchrony and the occurrence of migration obstacles, three populations were defined (Figure 1). Two of them overlapped main river basins (Vydra and Křemelná Rivers), and the third was located in a small tributary Švelský Stream, which was separated from the rest of the river system by an impassable migration barrier. Within populations, fourteen synchronised population units were established. The mean cross-correlation coefficient between sampling sites within synchronised population units ranged from 0.75 to 0.96.

3.3 Individual growth and age

The estimated age of brown trout varied from age-0 to age-7, and individuals belonging to the age-1 and age-2 groups were the most numerous. The individual growth of all age groups were significantly different (LMM II; $F_{3;1268}$=13.25, P<0.0001; Adj. P<0.0001) and decreased throughout ontogenesis. The variation of individual growth among the spatial units was strongly age dependent. For age-0 (Figure 2) and age-1 (Figure 3) this relationship was significant or corresponded approximately to the limit of significance (Table 2). In contrast, the differences in individual growth among spatial units for individuals older than two years were non-significant (Table 2).

4. Discussion

The individual growth of brown trout in the studied populations was generally lower than in other populations located in streams with comparable latitudes [34,35]. This is most likely a result of environmental conditions correlated with the altitude (e.g., climate, nutrients, physical stream characteristics; [36]) as well as endogenous (e.g., density) [4] and genetic factors [37]. The individual growth rate was age dependent, as it decreased along ontogeny, which is typical for brown trout [2,37,38]. The highest growth rate was found for the age age-0 class, most likely because small trout exhibit minimal foraging costs and a short satiation time [39]. In addition, juvenile fish also allocate a larger energy budget to structural growth [40], while older individuals display decreased growth, most likely as a result of reproduction costs [41,42] and increased lipid storage intensity [43].

In accordance with prior studies [20,44], differences in individual growth between populations divided by an impassable migration barrier were observed. This variations in individual growth might be due to differences in environmental conditions as well as in endogenous (e.g., density) and genetic factors between Švelský Stream and the Vydra River [37]. Nevertheless, in this study, significant variation in the individual growth of brown trout in a continuously passable small river basin was documented, similar to the pattern Lobon-Cerviá reported [45]. The differences in growth between spatial units were strongly age dependent. Individual growth varied significantly across all of the observed spatial units (sampling sites, synchronised population

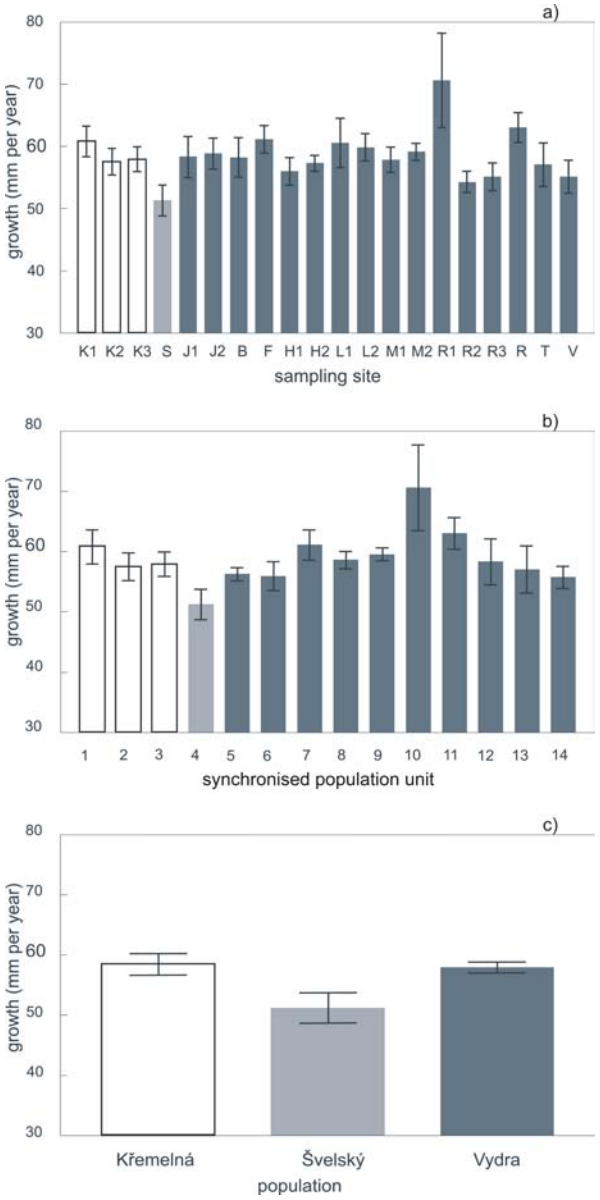

Figure 2. Annual individual growth of age-0 individuals across a) sampling sites; b) synchronised population units; c) populations (empty columns - population Křemelná; light filled column - population Švelský; dark filled columns - population Vydra). Values are adjusted means ± S.E.

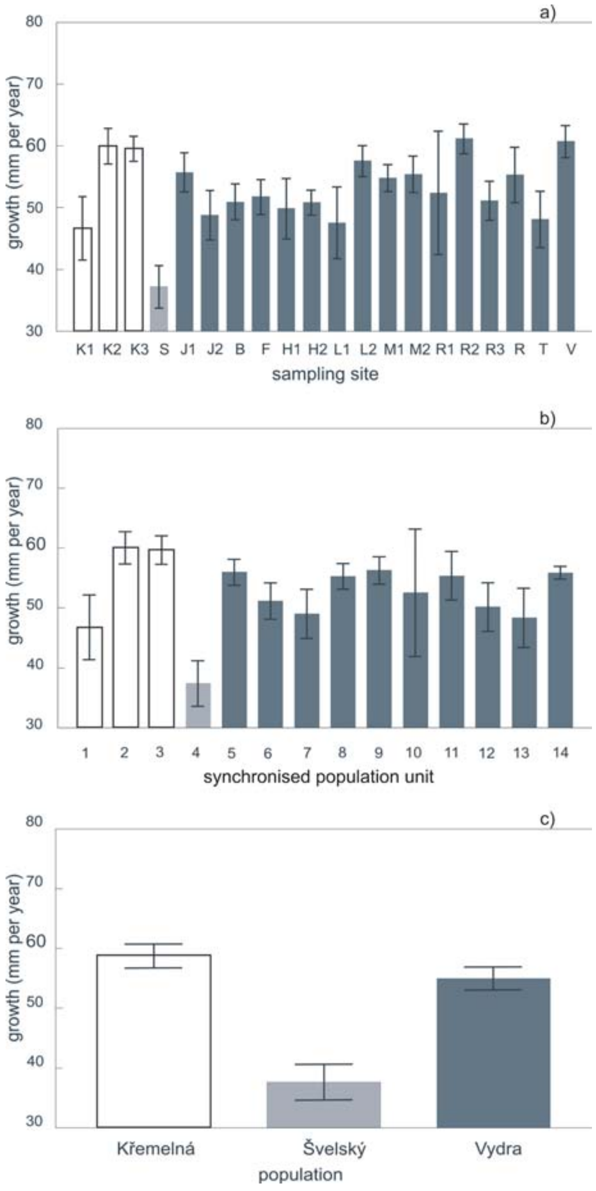

Figure 3. Annual individual growth of age-1 individuals across a) sampling sites; b) synchronised population units; c) populations (empty columns - population Křemelná; light filled column - population Švelský; dark filled columns - population Vydra). Values are adjusted means ± S.E.

units and populations), but only in age-1; for age-0, the differences were close to the limit of significance (the greatest difference was observed at the level of synchronised population units). For age-2 and age-3 and older, the variation in individual growth among spatial units was non-significant. As brown trout in the headwaters of rivers in the Czech Republic usually achieve maturity at age-2 [35], it can be concluded that the individual growth of the juvenile stock varied among the defined spatial units, while the individual growth of adults did not. The fact that the individual growth of the adult stock did not reflect their affiliation with the defined population units could be caused by their lower sensitivity to the differences in environmental conditions [46] or variations in intra- and interspecific competition in headwaters [2,38]. Similarly, the majority of the adult stock was found in the lower parts of the Otava River during most of the year, and these fish migrated to tributaries only for spawning (see Klementsen *et al.* [47]). This suggests that the time spent in the home

Age group	Spatial scale	Model No.	Result
age-0	sampling site	LMM III	$F_{19;663}=1.48$, $P>0.0839$
age-0	synchronised population units	LMM IV	$F_{14;663}=1.81$, $P>0.0339$
age-0	population	LMM V	$F_{2;664}=4.63$, $P>0.0100$
age-1	sampling site	LMM VI	$F_{19;480}=3.96$, $P<0.0001$
age-1	synchronised population units	LMM VII	$F_{13;480}=3.84$, $P<0.0001$
age-1	population	LMM VIII	$F_{2;483}=16.65$, $P<0.0001$
age-2	sampling site	LMM IX	$F_{19;142}=1.01$, $P>0.4536$
age-2	synchronised population units	LMM X	$F_{13;143}=1.12$, $P>0.3436$
age-2	population	LMM XI	$F_{2;148}=2.47$, $P>0.0878$
age-3 and older	sampling site	LMM XII	$F_{7;14}=1.57$, $P>0.2222$
age-3 and older	synchronised population units	LMM XIII	$F_{4;14}=0.55$, $P>0.7004$
age-3 and older	population	LMM XIV	$F_{1;14}=0.33$, $P>0.5731$

Table 2. Results of LMM of annual individual growth of all age groups across sampling sites, synchronised population units and populations.

stream may not be sufficiently long to induce spatial variations in growth rates.

5. Conclusions

Šumava National Park is part of the largest pristine natural area in central Europe and represents an important European centre of biodiversity, with many endangered and rare species and habitats, including the headwaters of the Otava River. Brown trout in the headwaters of the Otava River are found in demographically independent synchronised population units that differ in terms of individual growth. The distinctiveness regarding individual growth and demography indicate the suitability of these population units for use as independent management units [32]. Improved conservation management can also be achieved *via* more rigorous analyses of the relationships among synchronised population units in the sense of metapopulation dynamics [48]. The observed populations in the main river basins (Vydra and Křemelná Rivers) are essentially dependent on spawning migrants from downstream areas of the Otava River. Therefore, it can be suggested that wise management of hatchery fish stocking and the fisheries themselves [49,50] and the restoration of longitudinal river continuity [51] in downstream river stretches are necessary to achieve viability of the brown trout populations in Šumava National Park.

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PAPER VII

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Demogenetic structure of brown trout *Salmo trutta* Linnaeus, 1758 populations in mountain headwaters: implications for conservation management

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Summary

A demogenetic analysis based on 7 years of observation (2005–2011) was conducted to examine the population structure of brown trout *Salmo trutta* in pristine dendritic headwaters. The value of genetic divergence (F_{ST}) among sampling units ranged from −0.03 to 0.16. Demographic synchrony was low or moderate, and the average correlation coefficient of population growth between sampling units ($\bar{r}$) ranged from 0.28 to 0.66. No isolation by distance was observed, but genetic divergence was negatively correlated with demographic synchrony among sampling units. Variance in the population growth rate (i.e. local extinction probability) increased with distance from the mainstream and from other sampling units. In contradiction with the usual model of stream-dwelling salmonids, the upstream sections of headwaters holds only ephemeral subpopulations, whereas the mainstream played a role in the source area of the metapopulation. These findings stress the importance of the mainstream in management conservation for brown trout in low productive mountain headwaters.

Introduction

Demographic synchrony and analyses of genetic divergence have often been used to assess the spatial structure and history of fish populations (Hudy et al., 2010; Saura and Faria, 2011). When used separately, these methods are insufficient to reveal the population structure on a scale necessary for management decisions (Moran, 1953; Cattanéo et al., 2003; Wilson et al., 2004; Koizumi et al., 2008). The spatially explicit demogenetic models combining both methods (demographic and genetic) and spatial data, could bring an accurate insight into the population structure, but the body of such studies is still low, particularly in stream-dwelling fish, and new studies addressing this topic are needed (Frank et al., 2011; Vøllestad et al., 2012).

Stream-dwelling fish commonly occur in demographically and genetically independent units and connectivity of the watershed is critical for this differentiation (Henriques et al., 2010). Fish populations are often fragmented due to migration barriers (Letcher et al., 2007), which reduce or completely prevent migration (Northcote, 2010; Slavík et al., 2012). Isolation by distance (*sensu* Wright, 1943) has been also reported for many stream-dweling fish in migratory passable streams (Takacs et al., 2008; Griffiths et al., 2009; Lamphere and Blum, 2012). Interestingly, distinct subpopulations of stream-dwelling salmonid fish can exist within a narrow distance, ranging from hundreds of meters up to several kilometres (Carlsson et al., 1999; Vøllestad et al., 2012). Since such distances are often smaller than the migration capability of adult spawning migrants (Zimmer et al., 2010), the migration seems to be limited by behavioural constraints, which can cause a sharp differentiation among subpopulations (Neville et al., 2006; Östergren and Nilsson, 2011). Homing behaviour (Halvorsen and Stabell, 1990; Armstrong and Herbert, 1997), spawning site fidelity (Quinn, 2005) and limited movements induced by territorial behaviour (Höjesjö et al., 2007) might be factors reducing the flow of migrants between subpopulations of salmonid fish. Nevertheless, the intensity of behavioural constrains can differ between populations; their importance is closely related to the population life-history and the riverscape (Fagan, 2002; Östergren and Nilsson, 2011).

Griffiths et al. (2009) reviewed the three most common arrangements of population structure observed in brown trout *Salmo trutta*, considering individual behaviour and habitat connectivity. The first is panmixia, where spawning migration is not restricted and a gene-flow is intensive throughout the entire watershed. The second is a member-vagrant model, which suggests the existence of partially isolated stable populations with distinct gene pool (members), which are occasionally connected by straying individuals (vagrants). Finally, the third is a metapopulation model with partially isolated unstable subpopulations, where the extinction of a subpopulation and recolonization of available habitat occurs frequently. A source-sink, or the mainland-island metapopulation model has been suggested by many authors as the most common for stream-dwelling fish

(Dunham and Rieman, 1999; Gotelli and Taylor, 1999; Koizumi, 2011).

The brown trout population structure in Central Europe has been severely modulated by stocking management (Kohout et al., 2012). However, the genetic structure of populations at some localities seems to indicate at least a partial differentiation from an otherwise uniform genetic pattern of brown trout populations across Central Europe (Kohout et al., 2012). One location is the upper part of the Otava River situated in the protected area of the Šumava National Park (Kohout et al., 2012; Kohout, 2013). Conservation management in this area has had a significantly limited stocking and angling pressure on the brown trout population over the past two decades. A study of the structure and dynamic of this population can help to understand the recovery processes of salmonid fish populations under relaxed anthropogenic pressure. This study focused on the demographic and genetic structure of a wild brown trout population within a small headwaters system of this protected mountainous area. Specific aims of this study were to: (i) examine the demographic and genetic pattern of a brown trout population with respect to the connectivity of the headwaters system; (ii) determine basic management units for landlocked populations of brown trout occurring in the mainstream – tributary system.

Materials and methods

Study site

The study site was situated in a protected area of the Šumava National Park within the headwaters of the Otava River (49°1′N, 13°29′E) located in the Elbe catchment area of the Czech Republic. The Otava River arises after the confluence of the Vydra and Křemelná rivers (approximately 30 km long and 950 m a.s.l.) that both drain the basin area 224 km^2 (Fig. 1). The relief of the landscape in the region is mountainous and most of the streams originate above 1150 m a.s.l.; the watershed has a highly complex dendritic structure containing numerous tributaries and frequent branching. The studied streams are oligotrophic, with prevailing pristine conditions. Distribution of spawning grounds was equal among sampling sites, as the spawning grounds were spread out patchily throughout the system. The average flow at the sampling sites ranged from 0.01 to 2 m^3 s^{-1}. The substratum of streambeds was heterogeneous and contained sand, gravel, pebbles and boulders (Závorka et al., 2013). No physical obstacles prevent migration of the brown trout; the only exception was in the Švelský Stream, which contained a natural, impassable 2.5 m high steep boulder barrier located approx. 80 m from the confluence with the Vydra River. The boulder barrier prevents upstream migration to this tributary; however, downstream fish migration is possible. Sampling in the Švelský Stream was performed upstream of the migration barrier.

Sampling protocol

Twenty sampling sites were chosen along the longitudinal gradient of the study streams and their tributaries, according to an access permission issued by Šumava National Park (Fig. 1). Fish samplings were performed twice a year (in May and October) over seven consecutive years, from autumn 2005 to autumn 2011. In total, each sampling site was sampled thirteen times for fish abundance during the 7 years; the positions of the sampling sites did not differ throughout the study. To ensure the proper localization of the sampling sites during the study, the beginning and end of the sampled stream stretch were defined by GPS coordinates and by significant landscape elements (a bridge, rock, or large tree). Average length of the sampling sites was 200 m. Sampling effort did not change during the study and water quality during sampling (temperature and conductivity) did

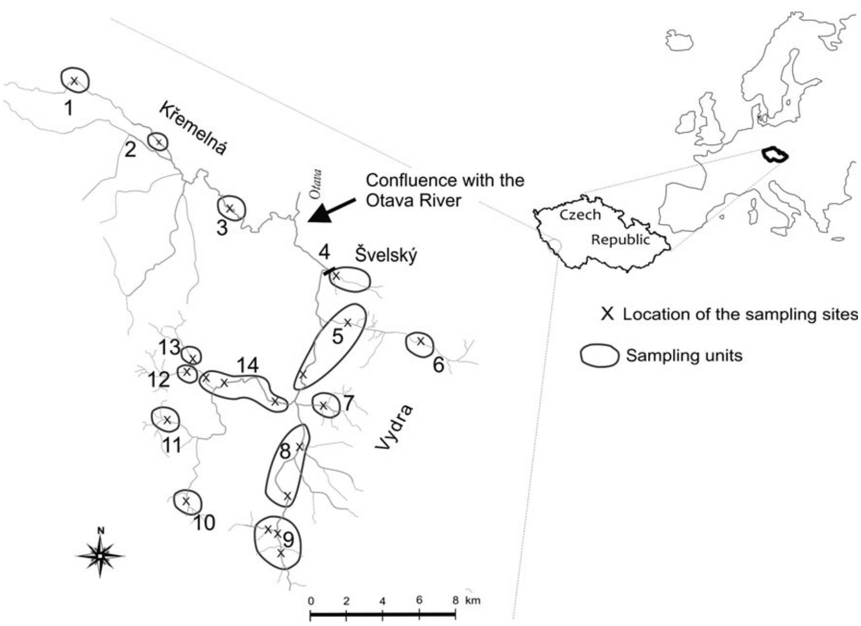

Fig. 1. Map of study area highlighting sampling sites, sampling units and streams

not vary substantially across the sampling events. Population density in sampled streams was generally low in all life stages including juveniles; therefore single pass electrofishing with a backpack electro shocker (EFKO, Germany) was used. This method is considered to be sufficient for the determination of brown trout abundance in mountain headwater streams with a low population density (Kruse et al., 1998; Cattanéo et al., 2003). Electrofishing is size-selective and can bring some bias in the estimation of individual abundance (Bohlin et al., 1989); however, the main goal of this study was to monitor population changes rather than to obtain absolute figures of fish abundance.

The dispersal of individuals was determined based on analyses of the mark-recapture programme published in the preliminary study (Závorka et al., 2013). The mark-recapture program was performed simultaneously with the each sampling of individual abundance (i.e. 13 times from autumn 2005 to autumn 2011). Each specimen was first anesthetized (2-phenoxiethanol) then individually tagged in the jaw using the Visible Implant Alphanumeric tags (Northwest Marine Technology, WA). Specimens of insufficient size for tagging with VIA tags (standard length smaller than 90 mm) were marked with VIE (visible implant elastomer) tags (Northwest Marine Technology).

Sampling of individuals for the purpose of the genetic analyses was performed in the autumn of 2005 and 2009. In total, samples from 188 individuals were collected (112 and 76 samples taken in the years 2008 and 2009, respectively). Randomly chosen individuals of various ages were sampled to reduce a potential bias in results due to the family effect and high concentration of the spawning immigrants from other river stretches during the autumn season. Chosen individuals were anesthetized using 2-phenoxy ethanol, and a tail fin clip of tissue was taken. Tissue samples were preserved in 96% ethanol.

Data handling

Density of brown trout at the spatial level of sampling sites was too low to provide reliable samples for genetic and demographic analyses; therefore, data from the sampling sites (both genetic and demographic) were pooled to larger spatial units and analysed as a one sample. As the basic spatial scale in this study was used 'synchronized population units' established in the preliminary study (Závorka et al., 2013). The 'synchronized population units' were delineated based on individual dispersal as determined by analyses of the mark-recapture programme and synchrony of individual abundance among the sampling sites during autumn (Závorka et al., 2013). Despite a relatively low overall recapture rate (9% on average), recaptured individuals displayed very low mobility and most were caught at the original sampling site (i.e. a stream stretch of an average 200 m length). Dispersal distance of individuals recaptured outside of their original sampling site was around 6 km (measured by channel distance). With respect to this finding, sampling sites were pooled to the 'synchronized population unit' when the correlation coefficient of individual abundance was higher than 0.56 and distance among them was lower than six km (for further details see Závorka et al., 2013). The 'synchronized population unit' number 10 was excluded from the present study, because the presence of brown trout at this locality was too low to provide a reliable dataset and could not be joined with any other 'synchronized population unit' due to its remote position. For higher clarity of results and discussion of this paper, the 'synchronized population units' are further categorized as 'sampling units'.

Based on previous results (Kohout et al., 2012; Kohout, 2013), nine clearly distinct polymorphic microsatellite loci (SsaD190, SsaD71, SSsp2213, SsoSL438, Str60, Ssa85, SSsp2216, Str73, SsoSL417) were chosen for analyses of genetic differentiation. Distribution of samples among sampling units is reported in Table 1. Sample size for genetic analyses at some sampling units in the present study was low, and worth noting that this might have introduced a random error in the results; therefore, the bootstrapping method was used to increase robustness of results.

The relative strengths of gene flow were investigated for each sampling unit by pairwise regression analysis addressing spatial distribution of the samples across the studied watershed. The genetic divergence was compared against the geographic distance in all pairs of sampling units using a one-dimensional stepping-stone model (Rousset 1997). Streams present linear elements with a different magnitude and geometry of branching, and Euclidian distance is not an optimal indicator of spatial distance in dendritic networks (Campbell Grant et al., 2007). We therefore used two measures of geographic distance in our models (Fig. 2). The first was a stream channel distance, calculated as a distance in meters between two sampling units following the centre of the stream channel; the measure was expressed in meters. The second measure was the number of dispersal nodes (i.e. incoming tributaries or dispersal corridors that are potentially suitable habitats for local populations). Tributaries with potentially suitable habitats were considered to be streams of 350 m minimal length. The number of steps separating

Table 1
Location of sampling units and numbers of individuals sampled for genetic analyses. Numbers and location of sampling units correspond to Fig. 1

Sampling unit	Location of the sampling unit	Number individuals sampled for genetic analyses
1	Křemelná River	7
2	Křemelná River	13
3	Křemelná River	13
4	Švelský Stream	27
5	Vydra River, Hamerský Stream	25
6	Hamerský Stream	5
7	Filipohuťský Stream	5
8	Modravský Stream	27
9	Březnický Stream, Luzenský Stream	17
10	Roklanský Stream	2 – excluded
11	Rokytka	9
12	Javoří Stream	11
13	Tmavý Stream	2
14	Roklanský Stream, Javoří Stream	24

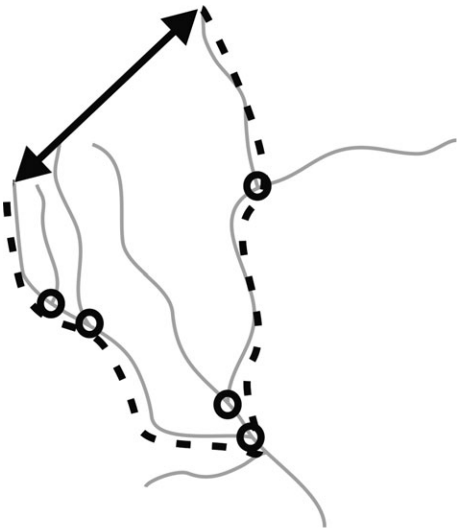

Fig. 2. Illustration of spatial measures used in the study. Arrow = shortest (Euclidian) distance between two locations. Dashed line = stream channel distance. Black open circles = dispersal nodes between locations

sampling units was than counted as a number of nodes (i.e. incoming tributaries) between the sampling units (Koizumi et al., 2006). All distance measures were based on the Basic Map for Water Management of the Czech Republic with a resolution scale 1 : 50 000.

Statistical analyses

The value of the fixation index (F_{ST}) calculated by software Arlequin 3.11 (Excoffier et al., 2005) was used as an indicator of genetic divergence between pairs of populations and sampling units. The statistical significance of F_{ST} was evaluated by 10 000 permutations using the confidence levels 0.001 and 0.05. Pearson's correlation coefficient of population growth rate was used as an indicator of demographic synchrony (r). Growth rate of the population was calculated as the change in population size over consecutive samplings deltaN (Green, 2003):

$$\mathrm{deltaN} = \log(\mathrm{N}+1)_{\mathrm{t}} - \log(\mathrm{N}+1)_{\mathrm{t}-1}.$$

The population growth rate was used instead of raw numbers of individuals, to avoid a bias in correlation toward a long-term trend in population size (Bjørnstad et al., 1999). Because of non-independence of the pairwise data, the Mantel test (10 000 permutations) was performed to verify the correlation between demographic synchrony, genetic divergence and spatial distance (i.e. Isolation by Distance models; Dinzin-Filho et al., 2013). Table-wide significance levels of genetic divergence and demographic synchrony were adjusted according to the sequential Bonferroni method (Rice, 1989).

Probability of local extinction was estimated as the variance in deltaN (Green, 2003), because the lack of individuals in a single sample is not a reliable indicator of local extinction due to possible limits of the survey methods (Gotelli and Taylor, 1999). The effect of the sampling units distribution on probability of local extinction was evaluated using a linear model with distance of the sampling unit from the Otava River (mainstream) and average distance of the sampling unit to other sampling units in the watershed as fixed factors. Model residuals were normally distributed for fixed factors expressed in both spatial measures (stream channel distance – Shapiro–Wilks test: P = 0.5574; number of dispersal nodes – Shapiro–Wilks test: P = 0.2191).

Results

Abundance of individuals was seasonal at all sampling units, with a higher abundance in autumn compared to the spring (Fig. 3). Genetic divergence among sampling units ranged

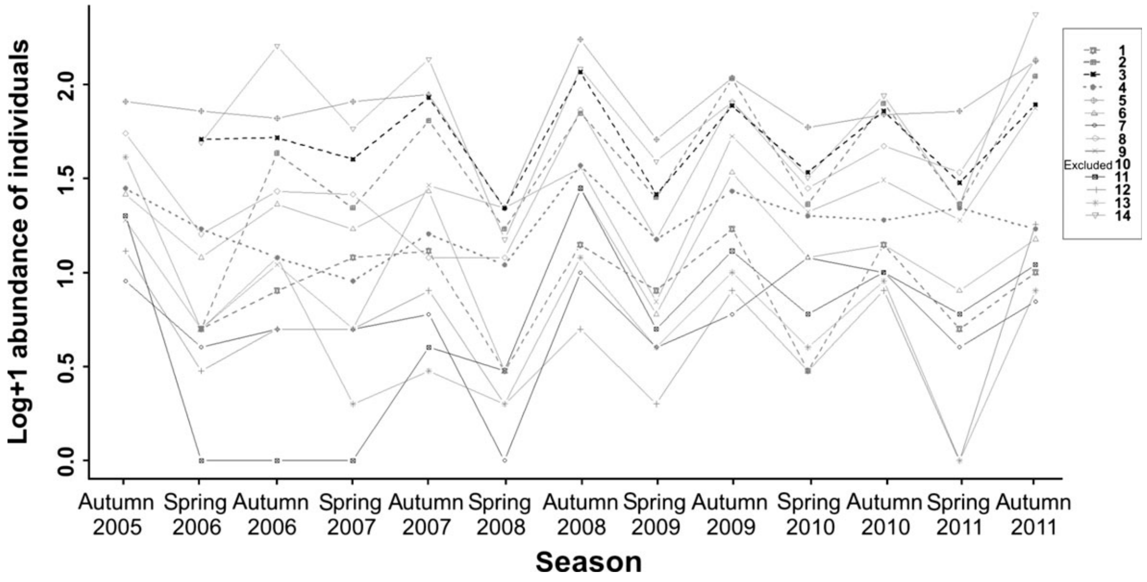

Fig. 3. Plot of individuals abundance in sampling units across 13 samplings between 2005 and 2011. Labels of sampling units correspond with Table 1 and Fig. 1

from −0.03 to 0.16, and 41 of 78 pairs of sampling units were significantly genetically divergent (Table 2). Most sampling units were also demographically independent; 61 pairs of sampling units displayed low correlation of population growth rate. Only 17 pairs were significantly demographically synchronized; however, eight of these pairs were genetically distinct (Table 2).

There was a negative correlation between genetic divergence (F_{ST}) and demographic synchrony (r) (N = 78, $R^2 = 0.385$, Mantel's P = 0.0049; Fig. 4). Hence, more demographically synchronized sampling units were more genetically related. After removal of the sampling unit in the Švelský Stream, which was isolated from the rest of the studied system by a migration barrier, the relationship remained unchanged (N = 66, $R^2 = 0.361$, Mantel's P = 0.0010; Fig. 4). There was no relationship in the geographic distance between sampling units and genetic divergence (F_{ST}) or the demographic synchrony (r) detected (i.e. no isolation by distance; Table 3). The lack of isolation by distance was indicated in all models independently whether the isolated sampling unit (Švelský Stream) was included in the data set. The lack of isolation by distance in the models was indicated by both measures of geographic distance (stream channel distance or dispersal node).

Variance of population growth rate deltaN (i.e. probability of local extinction) was positively related to the interaction of the sampling unit distance from the Otava River and average distance of sampling unit to other sampling units ($F_{1,9} = 7.19$; $P > 0.0251$; Fig. 5). This model was significant only when calculated with the number of dispersal nodes as a spatial measure; for stream channel distance this model was non-significant.

Discussion

Our study complemented an increasing but still weak body of studies addressing spatially explicit demo-genetic models of stream-dwelling salmonid populations (Koizumi, 2011; Saura and Faria, 2011; Vøllestad et al., 2012; Frank and Baret, 2013). Overall moderate genetic differentiation and demographic synchrony, lack of spatial gradient in genetic and demographic divergence (Wright, 1943 – isolation by distance) and independence of sampling units implies occurrence of isolated subpopulations within the observed watershed (Neville et al., 2006; Griffiths et al., 2009). Stability of subpopulations was tightly linked with their position against the main stream and other subpopulations in the system, which corresponds to the source-sink or mainland-island metapopulation model (Koizumi, 2011).

The studied system is relatively small (224 km^2), and all tributaries (with one exception) were migratory passable, therefore the adult spawning migrants should be capable of migrating throughout the entire watershed (Zimmer et al., 2010). The limited migration of individuals seems to be constrained by the number of dispersal nodes (Fagan, 2002) and behaviour of individuals rather than by physical migration obstacles (Östergren and Nilsson, 2011; Vøllestad et al., 2012). The lack of isolation by distance in the genetic and demographic gradient implies that population structure was not formed by only a

Table 2
Demogenetic structure of sampling units. Below the diagonal: coefficients of genetic divergence (F_{ST}); Above the diagonal: Pearson's correlation coefficients (r) of demographic synchrony

Sampling unit	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	******	0.757*	**0.755***	0.407	0.628	0.788*	0.117	0.447	0.45	–	0.564	0.445	0.478	0.443
2	0.03011	******	0.741*	0.437	0.656	0.637	0.304	**0.825***	**0.896***	–	0.487	**0.821***	0.603	**0.713***
3	**0.04103***	0.00231	******	0.667	0.872*	0.783*	0.393	0.573	0.589	–	0.782	0.523	0.644	0.656
4	0.06984*	0.06137*	0.03257*	******	0.68	0.541	0.501	0.456	0.355	–	**0.915***	0.233	0.528	0.087
5	0.04677*	0.02146*	0.00871	0.02484*	******	0.638	0.269	**0.730***	0.606	–	**0.775***	0.489	0.187	0.616
6	0.02186	0.03455	0.02344	−0.00713	0.00597	******	0.208	0.372	0.345	–	0.632	0.382	0.443	0.498
7	0.14742*	0.09167*	0.07996*	0.10855*	0.06982*	0.07302	******	0.282	0.296	–	0.425	0.167	0.292	0.093
8	0.05703*	**0.0394***	0.05342	0.03356*	**0.03286***	−0.0152	0.07739*	******	0.886*	–	0.526	0.801*	0.28	0.678
9	0.03011*	**0.03192***	0.03329*	0.03696*	0.02371*	−0.02253	0.0702*	−0.00087	******	–	0.256	0.728*	0.041	0.646
10	–	–	–	–	–	–	–	–	–	******	–	–	–	–
11	0.04954*	0.02975	0.03536*	**0.03288***	**0.0444***	−0.01901	0.08078*	0.00266	0.00863	–	******	0.293	0.619	0.379
12	0.01013	**0.04722***	0.05311*	0.03666*	0.04519*	−0.01135	0.14242*	0.02335	0.01369	–	0.01294	******	0.491	0.844*
13	−0.02619	−0.03131	−0.01477	0.03787	−0.01289	−0.03484	0.15808*	0.00011	−0.01743	–	−0.0128	−0.0325	******	0.545
14	0.02915*	**0.02279***	0.02353*	0.02596*	0.01973*	−0.0278	0.06267*	0.01004	0.00512	–	0.00238	0.00726	−0.00877	******

*Significant results (P > 0.05). Bold: pairs of sampling units having significant demographic synchrony and significant genetic divergence at the same time (Moran effect).

Table 3
Output of models addressing effect of geographic distance on demographic synchrony (r) and genetic divergence (F_{ST})

Model	Measure of geographic distance	Isolated sampling unit (Švelský stream) in the model	Model output
Geographic distance × demographic synchrony (r)	Number of dispersal nods	Excluded	N = 66, R^2 = 0.004, Mantel's P = 0.6241
	Number of dispersal nods	Included	N = 78, R^2 = 0.000, Mantel's P = 0.9326
	Stream channel distance	Excluded	N = 66, R^2 = 0.015, Mantel's P = 0.3522
	Stream channel distance	Included	N = 78, R^2 = 0.000, Mantel's P = 0.9658
Geographic distance × genetic divergence (F_{ST})	Number of dispersal nods	Excluded	N = 66, R^2 = 0.004, Mantel's P = 0.6241
	Number of dispersal nods	Included	N = 78, R^2 = 0.003, Mantel's P = 0.7719
	Stream channel distance	Excluded	N = 66, R^2 = 0.014, Mantel's P = 0.3522
	Stream channel distance	Included	N = 78, R^2 = 0.013, Mantel's P = 0.5446

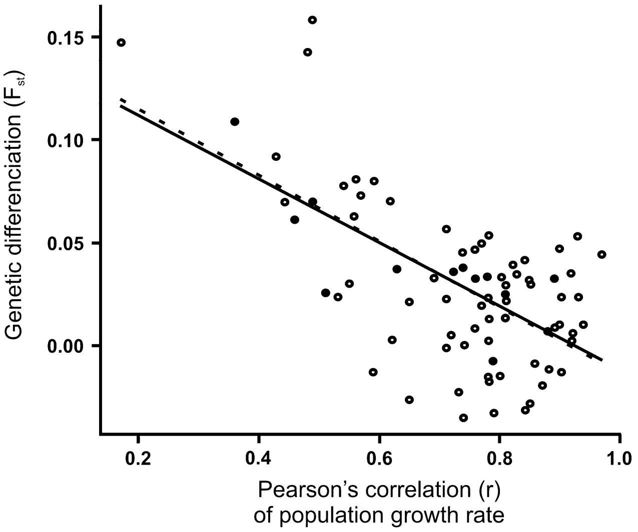

Fig. 4. Predicted values of the genetic divergence (F_{ST}) plotted against Pearson's correlation coefficient (r) of demographic synchrony among pairs of sampling units

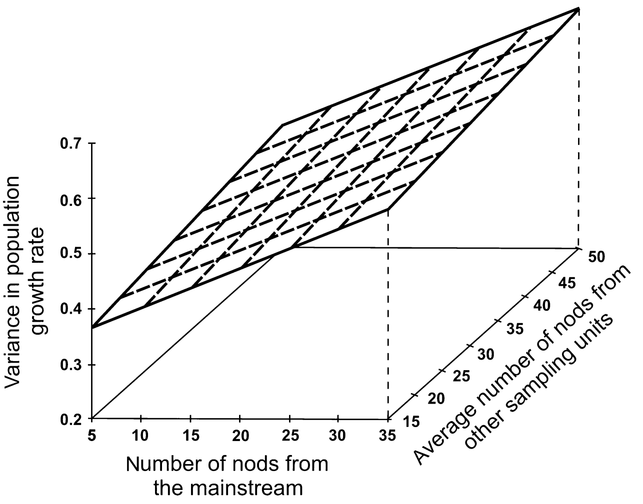

Fig. 5. Variance in population growth rate (deltaN) of sampling units plotted against distance of sampling unit from the mainstream (confluence of streams with the Otava River) and average distance of sampling unit to other sampling units within the studied system

dispersal of juvenile individuals from the spawning grounds (Einum et al., 2008) or a dispersal of straying spawners from their nursery spawning grounds (Garant et al., 2000). A more probable cause is the founder effect after the frequent recolonization and extinction in most upstream subpopulations that were strongly dependent on migrants from the mainstream (Koizumi et al., 2006; Østergaard et al., 2008; Griffiths et al., 2009). The sedentary prone behaviour of trout in higher elevated headwaters (Neville et al., 2006; Östergren and Nilsson, 2011) may also increase genetic and demographic isolation. The sedentary behaviour contrasts with the behaviour of mainstream migrants, who are more likely to occur in the lower parts of the watershed (Neville et al., 2006; Östergren and Nilsson, 2011). The difference in the elevation of headwaters and the confluence with the mainstream in the present study was around 200 m, which can lead to sharp differentiation in migratory behaviour of brown trout (Bohlin et al., 2001). Similar demogenetic population patterns as observed here could also arise due to artificial stocking (Koljonen et al., 1999); however, the studied population was protected and there was no artificial stocking in the observed watershed.

The present study illustrates that not only genetic analysis (Palsbøll et al., 2007), but also demographic data reflecting short-term tendencies are needed to define ecologically significant management units thoroughly (Crandall et al., 2000). Out of the general mode, a contradictory result of demographic and genetic analysis in eight pairs of sampling units was found, as the high level of the demographic synchrony did not respond to the significant genetic divergence among these sampled units. Demographic synchrony among populations was likely maintained by similar ambient conditions determining the carrying capacity of the environment (Moran, 1953; Cattanéo et al., 2003).

In summary, the headwaters of the Otava River were found to have a sink (island) habitat for subpopulations, while the mainstream (the Otava River) played the role of the source (mainland), which is in contradiction with the usual reproduction model of stream-dwelling salmonid fish (Klemetsen et al., 2003; Quinn, 2005). High isolation of tributaries in headwaters seems to reduce stability and population growth in the local subpopulations. Limited food resources and high energy requirements on individuals in mountain headwaters (Budy et al., 2008) increases juvenile mortality (Einum et al., 2008) and can increase cannibalism of eggs (Aymes et al., 2010) or juveniles (Finstad et al., 2006). Despite the low or temporarily negative population growth, the studied subpopulations genetically differentiated from the uniform genotype of lower situated parts of the watershed (Kohout et al., 2012; Kohout, 2013). The observed patterns highlight the importance of the sink subpopulation for recovery of genetic diversification (Frouz and Kindlmann, 2001) in populations of salmonid fish under relaxed anthropogenic pressure.

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