Growth-enhanced salmon modify stream ecosystem functioning

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Abstract: Use of fast-growing domesticated and/or genetically-modified strains of fish is becoming increasingly common in aquaculture, increasing the likelihood of deliberate or accidental introductions into the wild. To date, their ecological impacts on ecosystems remain to be quantified. Here, using a controlled phenotype manipulation by implanting growth hormone in juvenile Atlantic salmon (*Salmo salar*), we found that growth-enhanced fish display changes in several phenotypic traits known to be important for ecosystem functioning such as habitat use, morphology and excretion rate. Further, these phenotypic changes were associated with significant impacts on the invertebrate community and key stream ecosystem functions such as primary production and leaf-litter decomposition. These findings provide novel evidence that introductions of growth-enhanced fish into the wild can affect the functioning of natural ecosystems and represent a form of intraspecific invasion. Consequently, environmental impact assessments of growth-enhanced organisms need to explicitly consider ecosystem-level effects.

Keywords: Domestication, Ecosystem functioning, Escapees, Growth-Enhancement, Intraspecific variability, Stocking.

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Introduction

Rapid growth rate in plants and animals is a trait targeted extensively by humans for selective breeding and genetic modification to improve food production efficiency (Gjedrem *et al.*, 2012; Milla *et al.*, 2015). Salmonid fishes are extensively farmed for commercial production across the globe using selectively bred, fast-growing, domesticated phenotypes (Gross, 1998; Teletchea & Fontaine, 2014). Annually, large numbers of salmonids with varying degrees of domestication escape from commercial production and are also purposefully released into the wild for stock enhancement and conservation (Crawford & Muir, 2008; Lorenzen *et al.*, 2012; Sepúlveda *et al.*, 2013). Moreover, the production of genetically-modified salmonids using growth hormone (GH) transgenesis (Devlin *et al.*, 2015) represents a further source of growth-enhanced fish in the wild if accidental escapes occur. This is particularly true for Atlantic salmon (*Salmo salar*), one of the most widely produced salmonids in aquaculture (Glover *et al.*, 2017; Gross, 1998). To date, most investigations of growth-enhanced, domesticated salmonids in the wild have focused on their performance (Araki *et al.*, 2007; Fleming *et al.*, 2000; Sundt-Hansen *et al.*, 2012) and direct effects on wild conspecifics (Bolstad *et al.*, 2017; Fleming *et al.*, 2000; Glover *et al.*, 2017).

In salmonids, growth enhancement by selective breeding, GH-transgenesis and GH-treatment have produced qualitatively similar phenotypic effects on behaviour, physiology and life history (Devlin *et al.*, 2015; Sundström *et al.*, 2007b; Sundt-Hansen *et al.*, 2009). Among the effects accompanying enhanced growth are a higher movement activity likely associated with higher foraging activity, and a reduced antipredator behavior. Intraspecific variability is increasingly recognised as a key component of biodiversity with strong implications on ecosystem functioning (Des Roches *et al.*, 2017; Raffard *et al.*, 2018) and juvenile salmonids are key organisms of headwater stream ecosystems (Power, 1990). Therefore, the introduction of individuals with phenotypic changes caused by growth enhancement may represent a form of intraspecific invasion if the effects cascade across levels of biological organisation and affect prey communities and ecosystems, but this remains untested (Buoro *et al.*, 2016; Cucherousset & Olden, 2020; Devlin *et al.*, 2015).

Headwater stream food webs are fueled by benthic primary production and terrestrial resources and consumers on the top of the food web such as salmonid fish depend on a mix of prey from terrestrial and aquatic subsidies (Nakano & Murakami, 2001). Headwater stream

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food webs include epiphytic algae, terrestrial organic matter and bacteria at the base, grazer and decomposer invertebrates as primary consumers, and predatory invertebrates and fish as the secondary consumers and predators (Wetzel 2001; Vander Zanden et al. 2007). Experimental manipulation of fish in a Northern Californian stream has demonstrated a top-down control of food webs through a trophic cascade of predatory fish including juvenile steelhead trout *Oncorhynchus mykiss* (Power, 1990). More recently, experimental investigations have demonstrated the importance of intraspecific variability on ecosystem functioning in several freshwater fish species (Bassar *et al.*, 2010; Harmon *et al.*, 2009; Matthews *et al.*, 2016; Raffard *et al.*, 2021), highlighting that controlled phenotype manipulation can provide further insights into our understanding of how intraspecific variability affects ecological dynamics.

Here, we investigated the ecological effects of growth enhancement by stimulating growth of salmon using a non-heritable treatment (GH implants). The general aim was to determine the potential ecosystem consequences of growth-enhanced fish entering natural streams and understand the association between GH-induced phenotypic changes and ecosystem effects. Growth-enhanced salmon were produced by intraperitoneally implanting offspring of wild parents with GH, while sham-treated individuals were implanted with vehicle only (McLean *et al.*, 1997). This approach was selected because it presumably allows for the mimicking of heritable changes obtained through GH-transgenesis and breeding selection. As such, the independent assessment of the direct effects of rapid growth can be obtained while controlling for genetically correlated traits often modified during artificial selection (Devlin *et al.*, 2001). Specifically, we first tested the hypothesis that GH-treatment would induce significant changes on a suite of phenotypic traits. Second, we predicted that these changes would affect prey community structure and subsequently modify important ecosystem functions.

Materials and Methods

Experimental approach

Our experimental approach was based on the use of a series of three complementary experiments (Supporting Information: Figure S1). We first aimed at quantifying growth-hormone induced changes across levels of biological organisation, from individuals to community and ecosystems. Following this approach, we then aimed at identifying some specific changes in phenotypic traits that could be associated to the effects observed on community structure and ecosystem functioning. Therefore, an experiment was first conducted in 2015 with GH- and sham-treated individuals released into experimental streams to quantify the effects of GH-treatment on growth rate and body morphology of individuals and to determine the community and ecosystem consequences of growth-enhanced salmon . In 2016, two additional and complementary experiments were performed in small stream mesocosms to quantify the effects of GH-treatment on fish behaviour and nutrient excretion. Importantly, in parallel to these experiments, GH- and sham-treated individuals were maintained and fed *ad libitum* with commercial feed to quantify their phenotypes under hatchery conditions to serve as a baseline to assess change under stream conditions.

Experiments were conducted in 2015 and 2016 at the NINA Research Station at Ims in Norway (58º59’N, 5º58’E). In 2015, two experimental streams with natural substrate and water from the nearby Lake Liavatn were used (Supporting Information: Figure S1). They were divided into twelve sections, 23 m long and 0.75 m wide (17.02 ± 1.47 m^2). Lateral dividers of plastic mesh (4 × 4 mm) allowed flux of water and invertebrates. Longitudinal dividers of plastic sheets and galvanised steel mesh were inserted in the substrate to separate two parallel sections (Taylor, 2006) . Prior to the experiment, experimental streams were inoculated on 17 June 2015 with primary producers and invertebrates from the River Imsa (Supporting Information: Supplementary Methods).

In 2016, two additional experiments used 40 stream mesocosms (Supporting Information: Figure S1) made of fiberglass (4.5 m long × 0.25 m wide) containing natural gravel substrate. Stream mesocosms were paired structures, with a wooden or fiberglass wall creating two channels that shared the same water inlet from Lake Liavatn and grouped in blocks of four. A mesh (4 × 4 mm) was placed at the upstream and downstream ends of each mesocosm. To assess the behavioural effects, stream mesocosms (n = 20) were inoculated on 5-9 July 2015 with aquatic invertebrates collected from the River Imsa. In addition, four small boulders (approx. diameter 10 cm) covered with bryophytes and biofilm were added at 1.25 m, 2.75 m, 3.75 m, and 4.25 m within all mesocosms. To assess the consequences on nutrient excretion,, stream mesocosms (n = 20) were dried fully for two weeks prior to the experiment and water inflow reopened on 2 June 2016. The mesocosms were inoculated on 15 June 2016 with aquatic invertebrates and by adding two colonised cobbles collected from the River Imsa in each mesocosm. Water flow was regulated at approx. 2 L·s^{-1} to obtain a similar water depth of 11.16 ± 1.54 cm in all mesocosms and a covering net (mesh size: 15 × 15 mm) added to prevent potential bird predation.

Growth hormone treatment

Wild adult *S. salar* of the River Imsa were stripped and eggs artificially fertilized on 11 November 2014 (9 males, 16 females) and 9 November 2015 (33 males, 23 females). The eggs and subsequent juveniles were incubated in standard hatchery tanks until first feeding (9 March 2015 and 15 March 2016), when they were moved to feeding tanks (2 m^2). First-feeding juveniles were fed commercial feed (EWOS) *ad libitum* from automatic feeders. From 17-20 June 2015 and on 22 June 2016, juveniles were anaesthetized with Benzoak VET (www.europharma.no) (1.5 mL·L^{-1}) and fork length and weight measured to the nearest mm and 0.01 g, respectively. A small incision was made in the abdomen where an 8 mm passive integrated transponder (PIT) tag was inserted. Each fish was then randomly assigned to a treatment: GH treatment by implanting intraperitoneally with sustained-release recombinant bovine growth hormone (bGH; Posilac; Monsanto Company, St Louis, MO, USA) or sham treatment by implanting a corresponding volume of vehicle (sesame seed oil) using a Multipipette® M4 (Eppendorf, Hamburg, Germany). The GH treatment represented a dose of 1 mg bGH·g^{-1} fish biomass, previously shown to elicit a growth response (Raven *et al.*, 2012). Following recovery from the anaesthetic, fish were held in indoor tanks and fed commercial feed (EWOS) *ad libitum* before being used in the experiments. The use of GH-implant was selected because it had clear advantages over genotypic alternatives (e.g. GH-transgenic or breeding-selected aquaculture strains) to create a growth-enhanced phenotype which can be studied in semi-natural settings. For instance, the use of GH-transgenic would risk releasing them into nature and comparisons between GH-transgenic and non-transgenic conspecifics are problematic as age/size matching is impossible due to the different growth rates from hatching. The use of breeding-selected aquaculture strains and comparisons with wild individuals would be questionable because aquaculture selection has had several phenotypic targets over the generations (e.g. disease resistance, stress response) (Gross, 1998), making it difficult to identify which trait is important for ecological impacts.

Individual, community and ecosystem consequences of GH-treatment

In 2015, fish from both treatments (GH- and sham-treated) were introduced into the four most upstream sections of the experimental streams on 7 July (19 days after treatment to allow GH to act) (n = 2 section replicates per treatment, Supporting Information: Figure S1). Fifty individuals were placed in each section (n = 200) at a density of 2.94 ind$\cdot$m^{-2} that was within the range of natural densities observed in headwater streams in Norway (Teichert *et al.*, 2013). No fish were added in the four contiguous downstream sections. Fish were also placed in the four most downstream sections of the experimental streams. However, these sections were subsequently removed from analyses due to; i) high proportion of fish escaping from the parallel sections with a different treatment, and ii) the lasting ecological effects of GH-treatment along the upstream-downstream gradient (Supporting Information: Supplementary Methods and Figure S4). Before introduction, fish body mass was measured. GH-treated fish (4.51 ± 0.87 g) had significantly higher body mass than sham-treated fish (3.85 ± 0.91 g) at release ($F_{1,196} = 27.25$; $p < 0.001$) and higher growth rate (Table 1), indicating that GH-treatment enhanced growth. On 18 August 2015, fish were recaptured in the experimental streams by electrofishing (backpack mounted Geomega FA 4 apparatus, Terik Technology, Norway). Several electrofishing passes were carried out until no fish were caught in two consecutive passes. Recaptured fish were euthanized using an overdose of Benzoak VET (6 mL$\cdot$L^{-1}). In addition, 192 fish (96 GH-treated and 96 sham-treated) were maintained after tagging in eight hatchery tanks with flow-through water (60 L) and fed commercial feed (EWOS) *ad libitum* from automatic feeders until 22 August 2015. Four tanks contained GH-treated individuals and four tanks sham-treated fish (n = 24 per tank).

Individual consequences

Fish were scanned for PIT tags and measured for fork length and mass at the end of the experiments. We measured GH treatment effects on two phenotypic traits. Specific growth rate (SGR, %$\cdot$d^{-1}) was evaluated based on changes in body mass of individuals (Závorka *et al.*, 2017). Body shape of individuals was quantified by morphometric analyses of 14 landmarks selected from pictures of the left side of fish at the end of the artificial streams experiment (Závorka *et al.*, 2017). The first two non-uniform components of body shape variation (i.e. partial warps) were subsequently used to describe morphological differences (Supplementary Methods). These traits were quantified only for those individuals recaptured in the section where they were introduced.

Community and ecosystem consequences

Invertebrate community and ecosystem functions were measured at several locations (positions) within each section located along the upstream-downstream gradient. This was done to capture the spatial heterogeneity along the 23 m of each section and because the impact of growth-enhancement can vary along this gradient due to induced changes in microhabitat use (Sundström *et al.*, 2007a).

Invertebrates were collected using a standardised procedure with a Surber net (20 × 20 cm frame, 0.04 m^{2}, 500 µm mesh) allowing estimates of the density of each taxon (ind·m^{-2}) before fish recapture. Three samples (positions A, B and D; A being the most upstream position within each section) were collected within each section. Samples were stored in 90% ethanol and subsequently identified to the lowest taxonomic level (mainly Family) and counted under a microscope. Four families of invertebrates (Trichoptera, Diptera, Mollusca and Ephemeroptera) dominated the invertebrate community in the experimental streams. They belonged to seven families and several functional groups: Rhyacophilidae (free-ranging and strict predators), Polycentropodidae (filterers / predators feeding on invertebrates and organic debris), Hydropsychidae (omnivorous filter feeders and grazers with some predatory behaviour), Chironomidae (functionally diverse taxa composed of gatherers-collectors, shredders, grazers, predators and filter feeders), Planorbidae (strict grazers) and Baetidae (mixed grazers and gatherers-collectors) (Dudgeon & Richardson, 1988; Kjaerstad *et al.*, 2018) (Callisto *et al.*, 2007).

For ecosystem functioning, measuring devices were installed on 27 July and removed on 18 August 2015. Primary production was estimated by measuring standing algal biomass on ceramic tiles (10 × 10 cm). Tiles were installed along the upstream-downstream gradient position A, B, C, D and E. Total benthic chlorophyll-*a* concentration (µg chlo a·cm^{-2}) was measured using a portable fluorometer (BenthoTorch, BBE moldaenke GmbH, Germany) (Kahlert & McKie, 2014) and primary production expressed as a rate (µg chlo a·cm^{-2} day^{-1}). Three measurements were performed per tile to capture potential variability and averaged for subsequent analyses. The decomposition of organic matter was quantified by measuring leaf litter breakdown (Woodward *et al.*, 2012) (Supplementary methods). Decomposition rate (K, day^{-1}) was calculated using fine-mesh bags to assess microbial activity and coarse-meshed bags to assess invertebrate activity (Alp *et al.*, 2016; Lecerf *et al.*, 2005).

Behavioural and nutrient excretion consequences of GH-treatment

Behavioural effects

60 GH-treated and 60 sham-treated individuals were introduced into the stream mesocosms in groups of six individuals of matching size (n = 10 replicates per treatment, Supporting Information: Figure S1) on 9 July 2016. Individuals stayed in the stream mesocosms until the end of the experiment (August 3 2016), except during 18-19 July 2016 to measure open-field activity in different tanks (Supplementary Methods). At release, GH-treated fish (5.36 ± 1.19 g) had significantly higher body mass than sham-treated fish (4.72 ± 1.21 g) ($F_{1,118} = 8.65$; $p = 0.004$), indicating that the GH implant enhanced growth. Activity was quantified using open field tests (Supplementary Methods) that are a measure of undisturbed movement in a uniform homogenous environment providing estimates of activity in salmonids (Závorka *et al.*, 2016). Distance moved during the trial ($cm \cdot 10\ min^{-1}$) was used as a proxy of individual activity (Závorka *et al.*, 2016). Novement and habitat use were measured (Supplementary Methods) by determining individual longitudinal position within the stream mesocosms using active PIT telemetry (Cucherousset *et al.*, 2005). Individual movement within the stream mesocosms was quantified as a count of the number of 0.25-m sections between two consecutive positioning intervals. Habitat use was calculated as the probability of being detected within a 0.25 m section containing a boulder considering all positioning intervals when an individual was detected.

Effects on nutrient excretion

55 GH-treated and 30 sham-treated individuals were released into the stream mesocosms on 6 July 2016. This experiment was designed as a paired-block design with four treatments: sham-treated fish (sham, 6 individuals per mesocosm), GH-treated with the same fish density as the sham treatment and therefore higher biomass (GH, 6 individuals per mesocosm), GH-treated fish with the same fish biomass as sham-treated fish (GH-LD, 5 GH-treated individuals per mesocosm) and a treatment with no fish (NF). Each treatment was replicated 5 times. In addition, 18 GH-treated and 18 sham-treated individuals were placed in indoor hatchery tanks (60 L), with two tanks containing GH-treated (n = 9 per tank) and two tanks containing sham-treated (n = 9 per tank) fish. Two days before introduction, fish were measured, weighed and assigned to treatment. At release in the stream mesocosms, body mass

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was 4.21 ± 1.00 g, 5.14 ± 1.38 g, and 5.14 ± 1.14 g for the sham-, GH- and GH-LD-treated fish, respectively. GH-treated fish (from the GH and GH-LD treatments) had significantly higher body mass ($F_{2,82} = 6.00$; $p = 0.004$) than sham-treated fish, indicating that the GH implant enhanced growth. At release, the average total biomass in each mesocosm was 25.27 ± 0.08 g, 30.85 ± 0.03 g, and 25.69 ± 0.11 g for the sham-, GH- and GH-LD-treated fish, respectively. On 6 August 2016, fish were removed from the stream mesocosms. All fish survived and were recaptured. N and P excretion rates ($\mu mol \cdot h^{-1}$) (Villéger *et al.*, 2012) were quantified for individuals from the indoor tanks and from the stream mesocosms (4 and 6 August 2016, respectively) at the end of the experiment (Supporting Information: Supplementary Methods).

Ethical statement

The care and use of experimental animals complied with Norway animal welfare laws, guidelines and policies as approved by the Norwegian Animal Research Authority with licences No. 7616 and 9057.

Statistical analyses

We evaluated the ecological effects of GH treatment using mixed linear regression in a Bayesian framework. Models were implemented in the R package rstanarm (Goodrich & Gabry, 2017) with Bayesian inference realized via Stan (Stan Development Team, 2017) using Hamiltonian Monte Carlo sampling (HMC). We used non-informative prior distributions (t-student distribution with 7 degrees of freedom) for all regression coefficients of the models. By using non-informative priors, we assume that the effects sizes are *a priori* null and all information comes from the data only. Thus, the Bayesian procedure is considered as by preventing overestimation of effect sizes. For each model, we ran three parallel HMC chains and retained 10 000 iterations after an initial burn-in of 2000 iterations. Convergence of HMC sampling was assessed using Brooks-Gelman-Rubin diagnostics (Brooks & Gelman, 1998). We ran multiple models that included fixed effects and their interactions. Model comparisons were then conducted using the approximate leave-one-out cross-validation method (LOO) using the Loo package (Vehtari *et al.*, 2016). The best fitted models were chosen based on the LOO Information Criterion (LOOIC). LOOIC has the same purpose as the Akaike Information Criterion (i.e. lower is better), but also integrates uncertainty in the parameters. We also tested the goodness-of-fit of the best fitted models by using the predictive posterior check approach as implemented in the rstanarm package. Medians of effect sizes and credible intervals at 95% ($CI_{95\%}$, within brackets) without marginalizing random effects, indicating uncertainties in model parameters and posterior predictions, were reported. We evaluated the statistical significance by ensuring that the $CI_{95\%}$ did not overlap with 0. This was done by determining the proportion of posterior values with a sign different from the median, i.e. confidence that the parameter is positive or negative (hereafter, $P_{</>0}$). GH-treatment effects were considered significant using a threshold of 0.05.

Phenotypic effects

We tested whether GH-treatment induced phenotypic changes of salmon using all measured phenotypic traits during the three experiments. To test the effects of GH treatment on body mass and growth rate, treatment (GH or sham) was used as a fixed effect. We included a random effect on intercept using tank ID for the hatchery conditions and section nested in each channel for the experimental streams (Supporting Information: Table S1). For the other phenotypic traits, body mass was included in the models as a fixed effect to determine whether GH- and sham-treated fish differ in phenotypic traits irrespective of their mass. Effect of GH-treatment on morphology (two partial warps; Supporting Information: Supplementary Methods) was tested using two fixed effects (treatment and body mass at experiment end) and section nested in each experimental stream channel as a random effect on intercept. We evaluated the effect of GH treatment on fish activity using three fixed effects (treatment, scoring session (categorical variable: T_0, T_1 and T_2) and body mass at scoring (log-transformed)) with individual ID as a random effect on intercept. Diel movement and habitat use measured in the stream mesocosms were analysed using treatment, time of the day (categorical variable with eight levels) and body mass (log-transformed, value at T_0 for the first three tracking sessions and at T_1 for the last three tracking sessions) as fixed effects. Models also contained individual ID nested within tracking session, and stream mesocosm as random intercepts. Finally, to test the effects of GH-treatment on P and N excretion rates, models included two fixed effects (treatment and body mass measured at T_2 (log-transformed)) and a random effect on intercept through tank ID in hatchery conditions and stream mesocosm nested in block.

Community and ecosystem effects

For community (i.e. density of the main invertebrate taxa in Surber nets) and ecosystem (i.e. primary production, total and microbial decomposition rates), we used log-linear models with community responses (e.g. number of Baetidae) sampled in a Poisson distribution . The models included two fixed effects: treatment and position within the section (A to E). Channel (categorical variable with 2 levels) was used as a random effect on intercept. Differences between effects of each treatment (i.e. regression coefficients "treatment") at each iteration (extracted from HMC posterior values) were calculated. Statistical significance of these contrasting effects was evaluated by ensuring that the $CI_{95\%}$ of the differences measured did not overlap with 0.

Results

Phenotypic effects

GH-treated individuals grew faster than sham-treated individuals in hatchery conditions (GH effect = 0.61, $CI_{95\%}$ [0.21; 0.98], $P_{</>0} = 0.004$) while there was no significant different among individuals released in the experimental streams (Figure 1A-B and Table 1). We also observed differences in body morphology with GH-treated individuals having a more streamlined body shape than sham-treated individuals (second partial warp, GH effect = 0.007, $CI_{95\%}$ [0.002; 0.010], $P_{</>0} = 0.011$, Figure 1C, Supporting Information: Figure S2). GH-treated individuals had higher activity levels measured in open field tests than sham-treated individuals (GH effect = 282.40, $CI_{95\%}$ [-14.40; 577.49], $P_{</>0} = 0.030$, Figure 1D). GH-treated individuals moved more than sham-treated individuals, irrespective of the time of day (Figure 1E), had different habitat use and spent more time in sections containing boulders (GH effect = 0.10, $P_{</>0} \leq 0.039$, Table 1). GH treatment also induced a change in nutrient excretion rate. Specifically, GH-treated individuals had a lower phosphorous (P) excretion rate than sham-treated individuals (GH effect = -0.20, $CI_{95\%}$ [-0.40; -0.02], $P_{</>0} = 0.024$, Figure 1F) while nitrogen (N) excretion rate was similar between treatments (Table 1). P and N excretion rates did not differ between treatments for individuals maintained under hatchery conditions (Table 1).

Community and ecosystem effects

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We found that GH-induced phenotypic changes had significant effects on the invertebrate community in the experimental streams. Invertebrate density averaged 24853 ind·m^{-2} (± 10795 SD) in the sections of the experimental streams containing fish. Invertebrate community was dominated by two predatory taxa (14.82%; Polycentropodidae and Rhyacophilidae) and five taxa of primary consumers (85.18%; Chironomidae, Hydropsychidae, Planorbidae, Simuliidae and Baetidae). For most taxa, except Hydropsychidae and Planorbidae, the effect of GH treatment was position-dependent (Figure 2A-B and Table 2). For the predatory taxa, there was an overall significant increase in the density of Polycentropodidae (GH effect ≥ 0.58, $P_{</>0} = 0$ in position B and D) and a decrease in the density of Rhyacophilidae in the middle and downstream positions of the sections containing GH-treated individuals (GH effect ≤ -0.88, $P_{</>0} = 0$ in position B and D, Figure 2A-B and Table 2). These changes were associated with an overall increase in the density of primary consumers that was observed in the locations (GH effect ≥ 0.14, $P_{</>0} = 0$ in position B and D, Figure 2C and Table 2). Specifically, and although some of these changes vary between positions, we observed increased densities of Chironomidae and Hydropsychidae while the densities of Planorbidae, Simuliidae and Baetidae decreased in the sections containing GH-treated individuals and (Table 2, Supporting Information: Figure S3).

We then found that GH-treatment modified several key ecosystem functions in the experimental streams and these effects were observed to occur consistently in all positions within the sections (Figure 2D-F and Table 2). Sections with GH-treated individuals had significantly lower primary production than sections with sham-treated individuals (GH effect = -0.93, $CI_{95\%}$ [-1.27; -0.59], $P_{</>0} =0$). We also found that sections with GH-treated individuals had a significantly higher total decomposition (GH effect = 0.11, $CI_{95\%}$ [0.02; 0.21], $P_{</>0} = 0.011$) and lower microbial decomposition (GH effect = -0.22, $CI_{95\%}$ [-0.38; -0.06], $P_{</>0} = 0.006$) of leaf litter (Figure 2D-F and Table 2). We also found that some of these community and ecosystem effects existed in the contiguous downstream sections with no fish, as changes in invertebrate community and a significant decrease in primary production (GH effect = -1.04, $CI_{95\%}$ [-1.41; -0.66], $P_{</>0} = 0$) and an increase in total decomposition (GH effect = 0.09, $CI_{95\%}$ [-0.02; 0.19], $P_{</>0} = 0$) were observed in sections downstream of GH-treated individuals (Supplementary Results, Figure S4 and Table S1).

Discussion

In the present study, we show that growth enhancement obtained using growth-hormone implant induces significant changes in a suite of functionally-important phenotypic traits in juvenile salmon and significant effects on invertebrate community and ecosystem functioning (Figure 3). Growth outcomes of GH treatment were context-dependent as we found that they differed between the hatchery conditions and the experimental streams. They are likely a result of a trade-off between energy returns and costs of food acquisition, which differs between hatchery and natural conditions (Leggatt *et al.*, 2017; Sundström *et al.*, 2007b). This is consistent with previous studies showing that growth outcomes tend to decrease as environmental complexity increases and food availability decreases (Leggatt *et al.*, 2017; Sundström *et al.*, 2007b; Sundt-Hansen *et al.*, 2012). The effects of GH treatment on individual behavior and metabolism can be complex, but our results suggest that GH treatment might alter foraging activity (Sundt-Hansen *et al.*, 2009) and/or foraging motivation (Sundström *et al.*, 2007a). This was observed with GH-treated individuals being more active, moving greater distances and spending more time in sections containing boulders, which likely represent foraging patches, than sham-treated individuals. In the experimental streams, these changes might lead to a higher consumption of predatory Rhyacophilidae by GH-treated individuals and it has been reported that juvenile salmon consume more Rhyacophilidae than Polycentropodidae (Sánchez-Hernández *et al.*, 2013). GH treatment might have induced the greater consumption of Rhyacophilidae in our experiment through three potential mechanisms. First, as Rhyacophilidae are free-ranging predators (Dudgeon & Richardson, 1988), this foraging strategy might expose them to a higher predation risk by the more active and risk-taking GH-treated individuals. Second, GH treatment could modify the metabolism and energy demands of salmon, requiring that they select prey with higher energy content to sustain their higher needs (White *et al.*, 2016; Zandonà *et al.*, 2011). Third, GH-treated individuals were larger at release than sham-treated individuals and might have consumed larger prey, such as predatory Rhyacophilidae, because they were less gap limited in their prey selectivity. The consequent decreased density of Rhyacophiliae could explain the increased density of Polycentropodidae through release from competition or predation. Trophic interactions in stream communities are complex, and whereas these suggested mechanisms remain speculative, this study highlights the need to better identify the mechanisms linking phenotypic changes induced by growth enhancement to changes in prey density.

Decreased density of predatory Rhyacophilidae was associated with an overall increase in the density of primary consumers that was observed for several taxa individually including Chironomidae which was the most abundant invertebrate taxon. These results likely indicate that changes in the density of predatory invertebrates directly decreased the density of primary consumers through consumption as Rhyacophilidae have been reported to consume a high proportion of Chironomidae (> 80% of their diet in some cases) (Thut, 1969). In addition to these consumptive effects linking GH treatment to the abundance of primary consumers, there may have been non-consumptive effects elicited by GH-treated salmon that decreased the foraging activity of predatory invertebrates or changing their drifting behaviour and contributed to the increased abundance of primary consumers. Indeed, the presence of fish with novel foraging behavior may induce a change in the foraging behavior of invertebrates and subsequently affect ecosystem functioning (McIntosh & Townsend, 1996). In general, we observed that community effects of GH-treated individuals on invertebrates varied along the upstream-downstream gradient. Although it could not be determined here, these differences could be caused by differences in habitat use by growth-enhanced salmon along the upstream-downstream gradient (Sundström *et al.*, 2007a) and by differences in invertebrate community structure caused by variation in microhabitats. The impacts on the invertebrate community might be caused by mass-independent phenotypic differences related to behavior and foraging activity acting through consumptive and non-consumptive effects on predatory invertebrates that subsequently affect primary consumers.

The invertebrate community in the experimental streams was composed of different functional groups and the density of invertebrates was in the higher range of values observed in Norwegian streams (Fjellheim *et al.*, 1993; Kjaerstad *et al.*, 2018), indicating that they were representative of natural headwater streams. It is therefore likely that the ecosystem effects induced by GH-treated individuals were caused by a higher density of primary consumers leading to a higher consumption of leaf-litter and periphyton (Power, 1990; Rosemond *et al.*, 1993). While there were no strict shredders of organic matter among the sampled invertebrates, the Chironomidae family is composed of a large panel of species with variable feeding strategies (Kjaerstad *et al.*, 2018) and Chironomidae have be demonstrated to consume leaf litter in streams (Callisto *et al.*, 2007). It is also common to find a high proportion of Chironomidae in leaf bags (Allard & Moreau, 1986). In the present study, we observed Chironomidae in the leaf bags and also a visual pattern of leaf consumption at the end of the experiment that is typical of Chironomidae (Callisto *et al.*, 2007). Because Chironomidae were the most abundant taxa and are consumed by Rhyacophilidae, it is likely that the GH-treatment induced a change in the intensity of the trophic cascade through consumptive and/or non-consumptive effects, increasing global decomposition rates and, to some extent, decreasing primary productivity. The effect on primary productivity could have been reinforced by the increased density of Baetidae and limited effects of the increase in Planorbidae. Changes in nutrient availability caused by lower P-excretion of GH-treated individuals might, in addition, have contributed to the reduction in primary production and microbial decomposition (Vanni, 2002). The strength of these effects was confirmed by the fact that some of them were also measured in the contiguous downstream sections where no GH-treated fish had been introduced. Although this remains to be tested, these findings suggest that the ecological effects of GH-treated salmon extend spatially and we hypothesise that this was caused by changes in the composition of invertebrate drift that, in turn, was due to GH-induced changes in the density of invertebrates.

The ecological importance of intraspecific variability is now widely recognized and intraspecific variability caused by natural processes and/or human activities has been demonstrated to play an important role in ecological dynamics (Des Roches *et al.*, 2017; Palkovacs *et al.*, 2012; Raffard *et al.*, 2018). Yet, studies using direct and controlled manipulation of functionally-important phenotypic traits are still needed to better link intraspecific variability to ecosystem functioning (Raffard *et al.*, 2018) and our study represents a rare case of such manipulation. The effects of introducing growth-enhanced, domesticated, fish in wild ecosystems can be high and act across levels of biological organization. Therefore, even within the native range of the species, they should be considered as a form of intraspecific invasion based on the ecological impacts they could induce (Cucherousset & Olden, 2020). Therefore, growth-enhanced strains should not be used when attempting to rebuild or supplement natural populations and the functional risks to wild ecosystems of escaped cultured and/or genetically modified fish when performing environmental risk assessments should be explicitly considered.

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Contributions: J.C., L.E.S.-H., and K.H developed the overall research questions with contributions from J.I.J., I.A.F. and B.T.B. J.C., L.E.S.-H., K.H, L.Z., and M.B designed the experiments that were performed with R.L. and K.A.E.B. R.L., K.A.E.B, J.C. and L.Z. compiled the data. M.B. and L.Z. analysed the data. J.C. wrote the paper with contributions from L.E.S.-H., M.B., L.Z. and K.H and all authors contributed to revisions. J.I.J. was the a lead investigator and the coordinator of the BiodivErsA project SalmoInvade funding this study and sadly passed away.

Significance statement

Fish with fast growth rates are artificially selected primarily for food production and might be released voluntarily or accidentally in the wild. Here, we demonstrate that the introductions of salmons with growth-enhanced phenotypes can affect ecosystem functioning. Therefore, the introduction of domesticated, growth-enhanced organisms might affect all levels of biological organisation and these should be accounted for when assessing their potential environmental impacts.

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Table 1. Summary statistics of the phenotypic effects of GH treatment. Each phenotypic trait is listed as a variable, with information about the experimental conditions (ES : experimental streams, HC : hatchery conditions and SM : stream mesocosms) and the selected model (indicated using a superscript). P is the proportion of posterior values with a different sign than the median, i.e. confidence that the parameter is positive or negative (hereafter, $P_{</>0}$). Effects were considered significant when $P_{</>0} < 0.05$ (values in bold). When models with interactions were selected, comparisons are reported for each modality of the parameter.

Variables	**Exp.**	**GH effect** Median [95% CI]	**$P_{</>0}$**
Body mass	HC^1	2.93 [1.29; 4.60]	**0.002**
	ES^2	0.01 [-3.13; 3.50]	0.497
Growth rate	HC^1	0.61 [0.21; 0.98]	**0.004**
before release	ES^2	1.07 [0.44; 1.75]	**0.008**
after release	ES^2	-0.26 [-0.81; 0.35]	0.104
Morphology - Warp 1	ES^3	0.001 [-0.010; 0.010]	0.444
- Warp 2	ES^3	0.007 [0.002; 0.010]	**0.011**
Activity	SM^4	282.40 [-14.40; 577.49]	**0.030**
Movement	SM^5	0.10 [-0.01; 0.20]	**0.039**
Habitat use	SM^5	0.21 [-0.02; 0.44]	**0.038**
N-excretion	HC^6	0.07 [-0.22; 0.37]	0.282
	SM^7	0.03 [-0.19; 0.27]	0.386
P-excretion	HC^6	-0.12 [-0.75; 0.48]	0.310
	SM^7	-0.20 [-0.40; -0.002]	**0.024**

[1] $Y_i = \alpha + \beta_1 \times \text{Treatment}_i + \varepsilon_{\text{TankID}[i]}$ with $\varepsilon_{\text{TankID}[i]} \sim N(0, \sigma^2_{\text{TankID}})$; [2] $Y_i = \alpha + \beta_1 \times \text{Treatment}_i + \varepsilon_{\text{Channel:Section}[i]}$ with $\varepsilon_{\text{Channel:Section}[i]} \sim N(\mu_{\text{Channel}[i]}, \sigma^2_{\text{Channel}[i]})$; [3] $Y_i = \alpha + \beta_1 \times \text{Treatment}_i + \log(\text{bodysize}_i)$ with $\varepsilon_{\text{Channel:Section}[i]} \sim N(\mu_{\text{Channel}[i]}, \sigma^2_{\text{Channel}[i]})$; [4] $Y_i = \alpha + \beta_1 \times \text{Treatment}_i + \beta_2 \times \log(\text{bodysize}_i) + \beta_3 \times \text{scoring session}_i + \varepsilon_{\text{fishID}[i]}$ with $\varepsilon_{\text{fishID}[i]} \sim N(0, \sigma^2)$; [5] $Y_i = \alpha + \beta_1 \times \text{Treatment}_i + \beta_2 \times \text{TimeofTheDay}_i + \beta_3 \times \log(\text{bodysize}_i) + \varepsilon_{\text{Tracking}[i]} + \varepsilon_{\text{mesocosm}[i]}$ with $\varepsilon_{\text{Tracking}[i]} \sim N(0, \sigma^2_{\text{Tracking}})$ and $\varepsilon_{\text{mesocosm}[i]} \sim N(0, \sigma^2_{\text{mesocosm}})$; [6] $\log(Y_i) = \alpha + \beta_1 \times \text{Treatment}_i + \beta_2 \times$

$\log(\text{bodysize}_i) + \varepsilon_{TankID[i]}$ with $\varepsilon_{TankID[i]} \sim N(0, \sigma^2_{TankID})$; [7] $\log(Y_i) = \alpha + \beta_1 \times \text{Treatment}_i + \beta_2 \times \log(\text{bodysize}_i) + \varepsilon_{block:mesocosm[i]}$ with $\varepsilon_{block:mesocosm[i]} \sim N(\mu_{block[i]}, \sigma^2_{block[i]})$.

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Table 2. Summary statistics of the community and ecosystem effects of GH treatment in experimental streams. Statistical analyses were performed for the upstream sections where GH- and sham-treated individuals were introduced. The selected model is indicated using a superscript. P is the proportion of posterior values with a different sign than the median, i.e. confidence that the parameter is positive or negative (hereafter, $P_{</>0}$). Effects were considered significant when $P < 0.05$ (values in bold). When models with interactions were selected, comparisons are reported for each modality of the parameter. A, B, C, D, and E represent the positions in the sections.

Level	**Variables**	**GH Effect Median [95% CI]**	$\mathbf{P_{</>0}}$
Community	Polycentropodidae^1	A: -0.33 [-0.36; -0.30]	**0**
		B: 0.58 [0.53; 0.62]	**0**
		D: 0.65 [0.6; 0.7]	**0**
	Rhyacophilidae^2	A: 0.15 [0.05; 0.24]	**0.001**
		B: -0.88 [-1.02; -0.72]	**0**
		D: -1.10 [-1.26; -0.94]	**0**
	Primary consumers (total)^1	A: -0.04 [-0.06; -0.03]	**0**
		B: 0.14 [0.12; 0.16]	**0**
		D: 0.61 [0.59; 0.63]	**0**
	Chironomidae^1	A: -0.30 [-0.32; -0.28]	**0**
		B: 0.40 [0.37; 0.42]	**0**
		D: 0.98 [0.96; 1,00]	**0**
	Hydropsychidae^1	A: 0.21 [0.17; 0.26]	**0**
		B: -0.06 [-0.12; 0]	**0.028**
		D: 0.21 [0.15; 0.28]	**0**
	Planorbidae^2	-0.39 [-0.42; -0.35]	**0**
	Simuliidae^1	A: 1.61 [1.55; 1.68]	**0**
		B: -0.71 [-0.82; -0.6]	**0**
		D: -1.49 [-1.6; -1.39]	**0**
	Baetidae^1	A: 0.62 [0.51; 0.72]	**0**

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		B: -0.97 [-1.15; -0.8]	**0**
		D: 0.02 [-0.18; 0.22]	0.587
Ecosystem	Primary production (log)[2]	-0.93 [-1.27; -0.59]	**0**
	Total decomposition[2]	0.11 [0.02; 0.21]	**0.011**
	Microbial decomposition[2]	-0.22 [-0.38; -0.06]	**0.006**

[1] $Y_i \sim \text{Poisson}(\lambda_i)$; $\log(\lambda_i) = \alpha + \beta \times \text{Treatment}_i + \gamma \times \text{Position}_i + (\delta \times \text{Treatment}_i \times \text{Position}_i) + \varepsilon_i$ with $\varepsilon_{\text{Channel}[i]} \sim N(0, \sigma^2)$; [2] $Y_i \sim \text{Poisson}(\lambda_i)$; $\log(\lambda_i) = \alpha + \beta \times \text{Treatment}_i + \gamma \times \text{Position}_i + \varepsilon_i$ with $\varepsilon_{\text{Channel}[i]} \sim N(0, \sigma^2)$; [3] $\log(Y_i) = \alpha + \beta \times \text{Treatment}_i + \gamma \times \text{Position}_i + \varepsilon_i$ with $\varepsilon_{\text{Channel}[i]} \sim N(0, \sigma^2)$

Figure captions

Figure 1. GH-treatment effects on Atlantic salmon (*Salmo salar*) phenotypic traits. Values are reported for sham-treated (black symbols) and GH-treated (blue symbols) individuals: (**A**) body mass (g) and (**B**) growth rate (SGR, $\%\cdot day^{-1}$) in hatchery conditions (*left*) and in the experimental streams (*right*) ; (**C**) morphology (second partial warp) as a function of body mass (g) in the experimental streams; (**D**) activity (open field test at T_0, $cm\cdot 10\ min^{-1}$) as a function of body mass (g); (**E**) habitat use (probability of being in a section with boulders) in the stream mesocosms; (**F**) P excretion rates ($\mu mol.h^{-1}$) as a function of body mass (g) in the stream mesocosms. (**A** and **B**) Posterior predictive distributions (median, 95% and 50% Posterior Predictive Intervals; thin and thick solid lines, respectively; without marginalizing random effects) are displayed for each treatment; (**C** - **F**) solid curve and dashed lines illustrate the median and surrounding 95% predictive intervals for each treatment, respectively. Open circles represent the observed values. * denotes significant effects with $P_{</>0} < 0.05$.

Figure 2. GH-treatment effects measured at the community and ecosystem levels. Invertebrate density ($ind\cdot m^{-2}$): (**A**) predatory Polycentropodidae; (**B**) predatory Rhyacophilidae; and ecosystem functions : (**C**) total primary consumers. (**D**) primary production (μg chlo $a\cdot cm^{-2}\cdot day^{-1}$); (**E**) total decomposition (day^{-1}); (**F**) microbial decomposition (day^{-1}). Values are reported for each position (from upstream to downstream) within the sections of the experimental streams containing sham-treated (black symbols) and GH-treated (blue symbols) individuals. Posterior predictive distributions (median, 95% and 50% Posterior Predictive Intervals; thin and thick solid lines, respectively; without marginalizing random effects) are displayed for each treatment and each position. Open circles represent the observed values. * denotes significant effects with $P_{</>0} < 0.05$.

Figure 3. Overview of the ecological effects of GH treatment of Atlantic salmon (*Salmo salar*) at the individual, community and ecosystem levels. Figure numbers correspond to the effects presented in the study.

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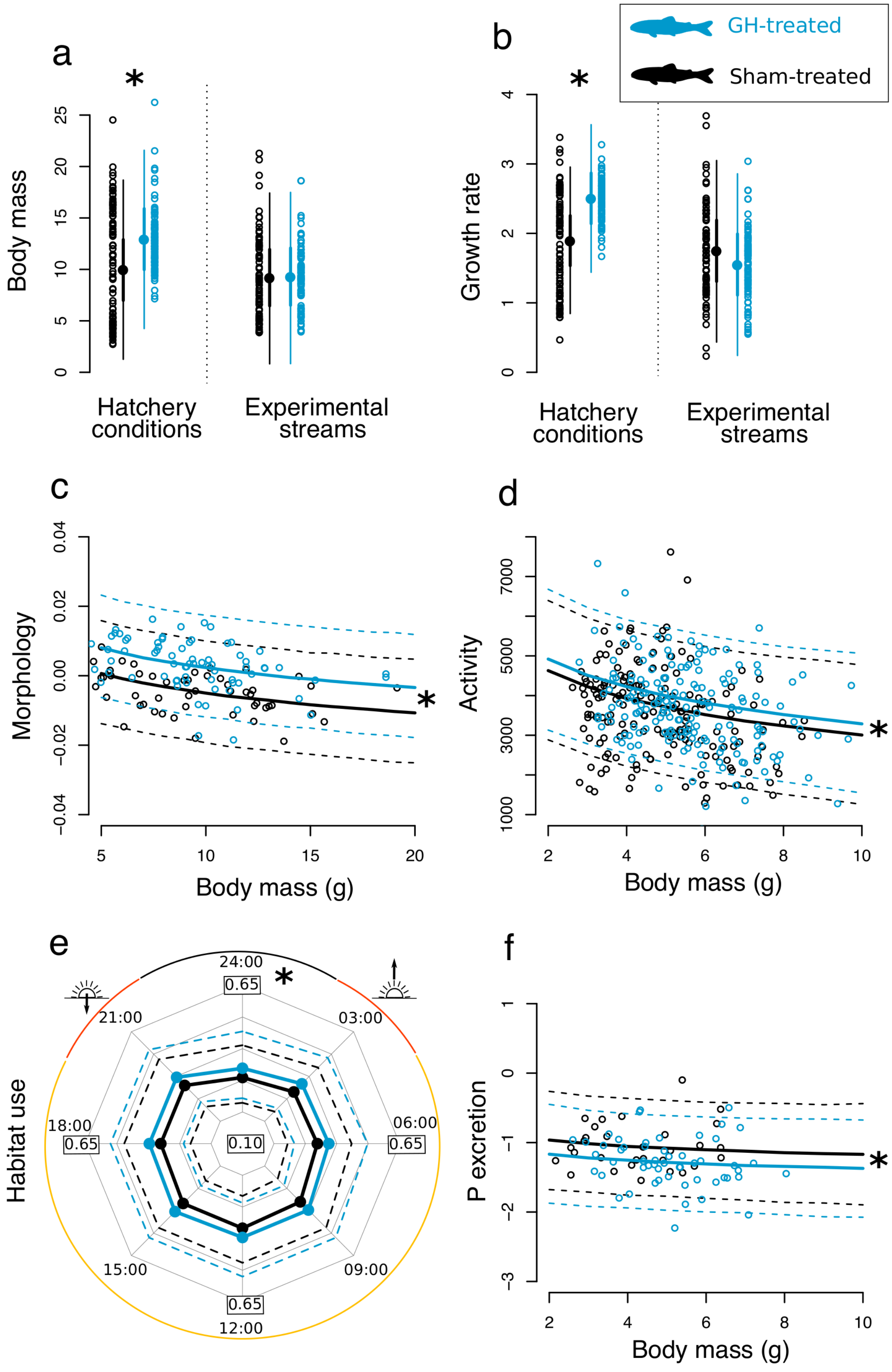

Figure 1

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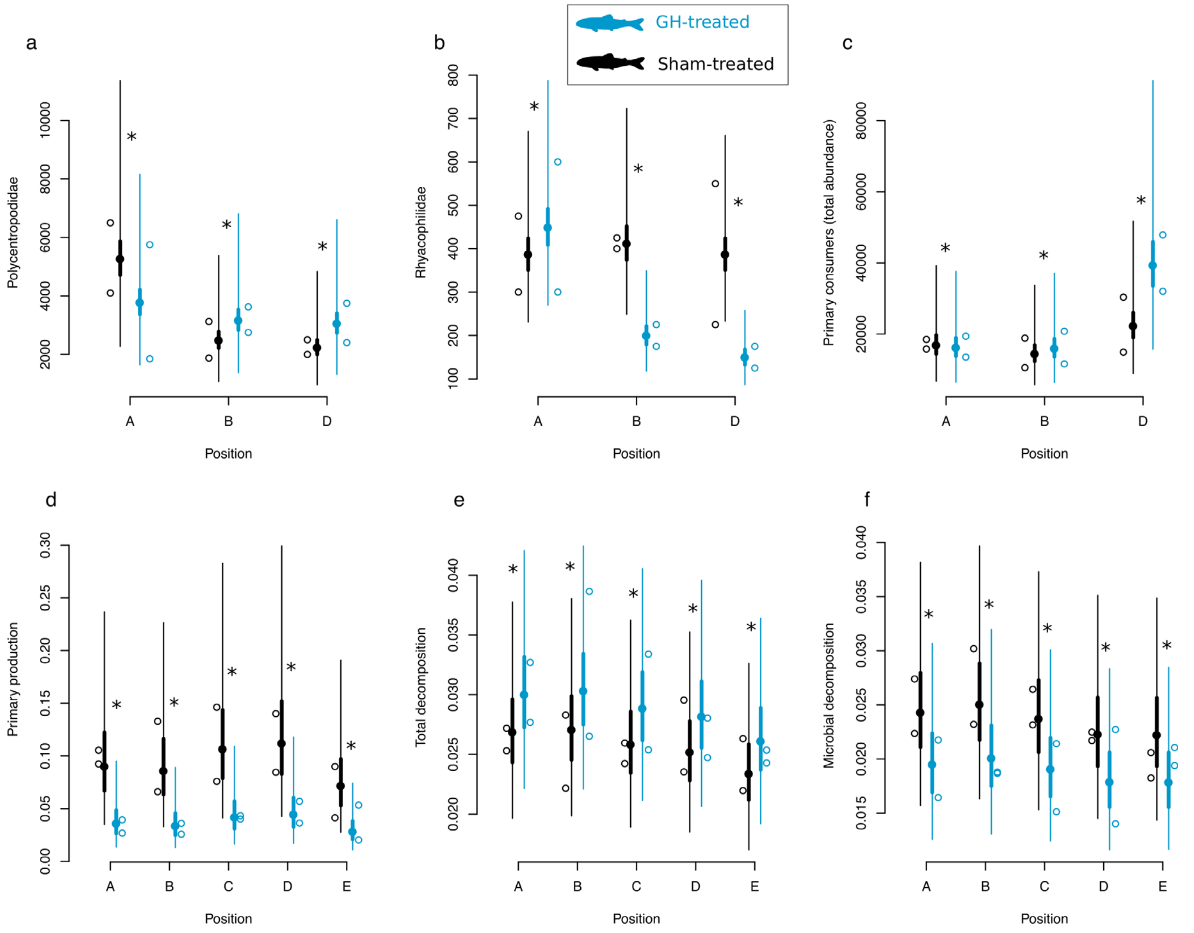

Figure 2

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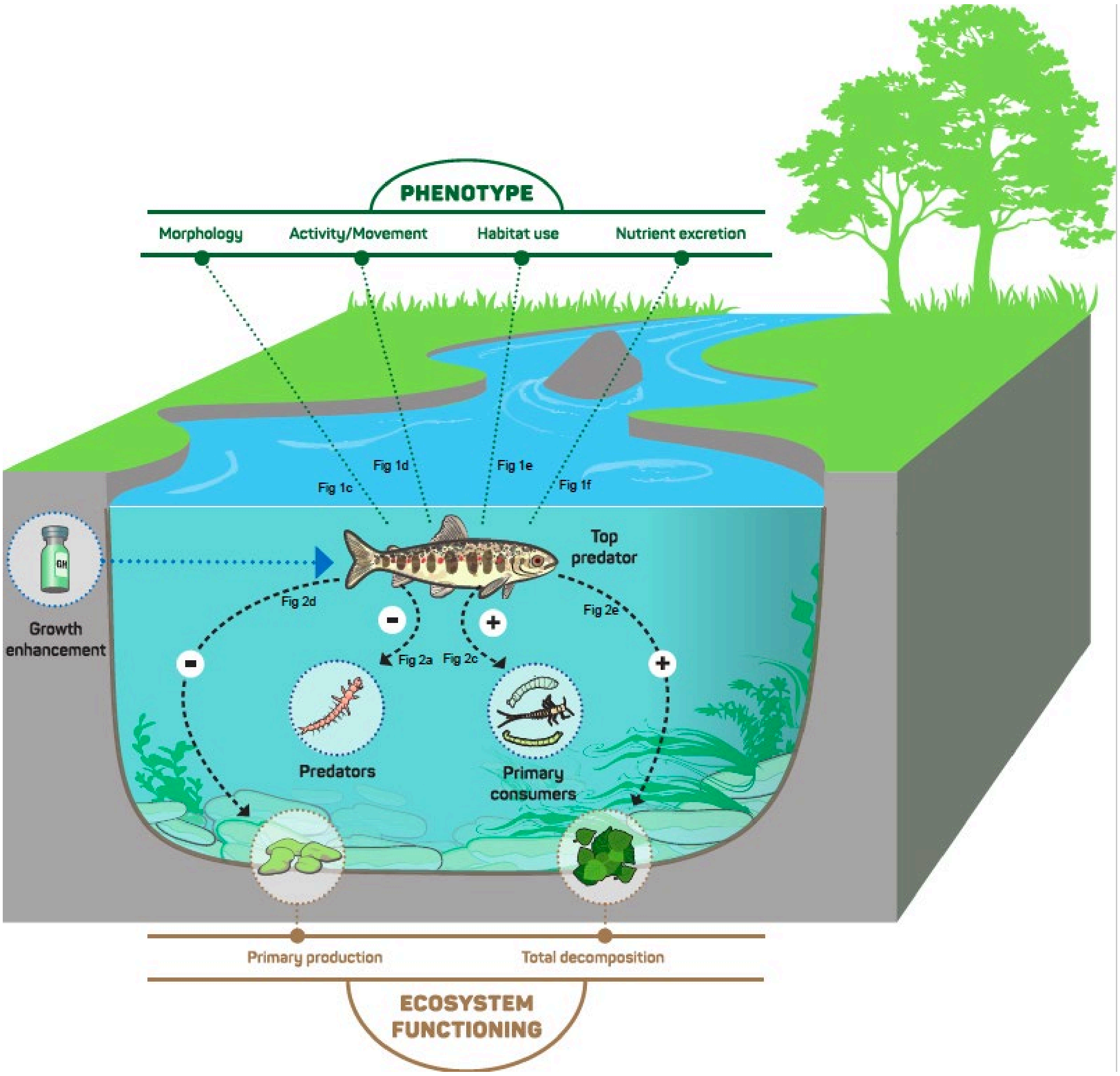

Figure 3

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