Life history genomic regions explain differences in Atlantic salmon marine diet specialization

Tutku Aykanat[1], Martin Rasmussen[2], Mikhail Ozerov[3,4], Eero Niemelä[5], Lars Paulin[6], Juha-Pekka Vähä[7], Kjetil Hindar[8], Vidar Wennevik[9], Torstein Pedersen[2], Martin-A. Svenning[10*], Craig R. Primmer[1*]

1 Organismal and Evolutionary Biology Research Programme, University of Helsinki, Finland. 2 Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Tromsø, Norway. 3 Department of Biology, University of Turku, Finland. 4 Kevo Subarctic Research Institute, University of Turku, Finland. 5 Natural Resources Institute Finland (Luke), Oulu, Finland. 6 Institute of Biotechnology, University of Helsinki 7 Association for Water and Environment of Western Uusimaa, Lohja, Finland. 8 Norwegian Institute for Nature Research (NINA), Trondheim, Norway. 9 Institute of Marine Research (IMR), Bergen, Norway. 10 Norwegian Institute of Nature Research (NINA), Arctic Ecology Department, Tromsø, Norway. * denotes equal share senior author.

Abstract:

An ecological consequence of climate change is the alteration of food-web structures. Species with ontogenetic (age-dependent) diet variation, such as Atlantic salmon (*Salmo salar*), may exhibit an age-dependent response to food-web perturbations, which may subsequently influence the demographic structure. We previously showed that age at maturity in Atlantic salmon is primarily influenced by few genomic regions (*vgll3* and *six6*), but whether these regions are linked to diet is unknown. We hypothesized that genetic variation in these life history genomic regions govern age-dependent resource utilization efficiency, which would subsequently influence age at maturity. To test this, we first performed stomach content analysis of Atlantic salmon sampled at sea on their return migration to fresh water, followed by targeted genotyping by sequencing. Here, we first showed that Atlantic salmon change their feeding strategies along their ontogeny. Consistent with the so-called feast and famine strategy, older age groups retained a heavier stomach content, which however came at the expense of running on empty more often. Next, we presented evidence that stomach fullness in Atlantic salmon is associated with *six6*, a gene previously shown to be potentially under divergent selection and correlated with age at maturity among populations. There was no association with *vgll3*, a gene with a large effect on sea age at maturity. Prey composition was marginally linked to both *six6* and *vgll3*. Our results suggest that Atlantic salmon individuals are not as generalist as previously thought and that genetic variation partly underlies resource utilization variation among individuals. Given that feeding strategies differ ontogenetically, and a spatially divergent genomic region is associated with diet acquisition variation, we predict populations with diverse maturation age will have diverse evolutionary responses to future changes in marine food-web structures.

Introduction:

Diet acquisition is a strong evolutionary force that can shape population demography and abundance, and is an integral determinant of ecosystem functions (Engen and Stenseth 1989, Svanback and Persson 2004, Bolnick and Araujo 2011). Individuals exhibit differences in prey preference and prey acquisition efficiency, which, if heritable, may be a target of selection and ultimately promote ecological specialization (Fox and Morrow 1981, Smith and Skulason 1996, Devictor et al. 2010, Elmer et al. 2010, Machovsky-Capuska et al. 2016, Sexton et al. 2017). Large-scale disturbances in community structure, e.g., as a result of climate change (Sydeman et al. 2015) alter food web structures and the composition of available resources (Daufresne et al. 2009, Pershing et al. 2015, Bentley et al. 2017), forcing species to rapidly adapt to new diet landscapes. Therefore, understanding the underlying mechanisms shaping food acquisition strategies is fundamental to evolutionary biology and vital for predicting species survival in a changing world.

If heritable, the inter-individual variation in resource acquisition strategies may have complex evolutionary consequences mediated by trade-offs between energy gain and survival across density- and frequency-dependent fitness landscapes (Mousseau et al. 2000, Reznick and Ghalambor 2001, Reznick 2016, Sexton et al. 2017). For example, increased boldness to improve resource acquisition success may come at the expense of higher predation risk, the fitness costs of which may be linked to predator densities (Gotthard 2000, Carter et al. 2010, Bolnick et al. 2011). Likewise, the composition and abundance of available resources may alter the demographic structure of a population (e.g., Heino and Kaitala 1999, Enberg et al. 2012). For example, fast growth at an early age, e.g. as a result of abundant food sources during the initial stages of life, may result in early maturation and hence a younger age at reproduction. In contrast, resource limitation due to high population densities results in increased allocation to somatic growth to improve size-dependent intrapecific competition (e.g., Reznick and Endler 1982).

Ontogenetic diet shifts in organisms may be viewed as a special type of resource acquisition strategy in which diet variation is expressed as a function of age. Ontogenetic diet shift is a significant source of variation in species' diet breadth, especially among size- and age-structured organisms, such as fishes. In general, relatively large and/or old individuals shift towards feeding at higher trophic levels and/or on larger prey items to maintain a positive energy balance (Werner and Gilliam 1984, Mittelbach and Persson 1998, Jensen et al. 2012). Under changing food-web dynamics, diet specialization among

different age groups may substantially influence the demographic structure and life history diversity (Sanchez-Hernandez et al. 2019). For example, changes in resource composition that favour younger age groups would improve growth and subsequently increase the rate of maturation and the probability of survival at early ages. Ontogenetic diet shift is associated with a suite of changes in an individual's morphology, physiology and behaviour to maximize the efficiency of particular resources at a given ontogenic stage, perhaps at the expense of reduced efficiency at other stages (Claessen and Dieckmann 2002). If ontogenetic diet variation has a genetic basis, then we predict some individuals in a population to have a genetic predisposition for high prey acquisition efficiency early in their life history (via physiological or morphological trade-offs towards efficient exploitation at earlier stages), which may be associated with compromised energy acquisition at later stages in life (Claessen and Dieckmann 2002). We predict that such genetically driven trade-offs in resource acquisition efficiency between early and late stages mediate the age structure and abundance within and among populations and maintain genetic variation in resource acquisition strategies, but such examples in the wild are rare.

Atlantic salmon (*Salmo salar*) is a fish species recognized as a diet generalist and an opportunistic feeder with extensive ontogenetic and stage- and space-structured individual variation in diet breadth (Erkinaro et al. 1997, Jacobsen and Hansen 2001, Haugland et al. 2006, Hvidsten et al. 2009, Rikardsen and Dempson 2010, MacKenzie et al. 2012). At sea, where most growth occurs, salmon increasingly feed on prey at higher trophic levels as they grow and age (Jacobsen and Hansen 2001, Rikardsen and Dempson 2010). The time salmon spend at sea prior to maturation (sea age at maturity) also varies greatly within and among populations (Friedland and Haas 1996). Although the functional and physiological basis underlying age at maturity is not entirely known, it is considered to be a threshold trait, whereby higher lipid deposition is associated with early maturation (Friedland and Haas 1996, Jonsson et al. 1997, Thorpe et al. 1998, Taranger et al. 2010, Jonsson and Jonsson 2011). Therefore, variation in resource acquisition may be a strong determinant of the life history variation in salmon and a vector via which natural selection can act and result in adaptive genetic changes in populations.

In Atlantic salmon, two genomic regions on chromosomes 9 and 25 have been shown to have a disproportionate influence on life history strategy and population differentiation within and among populations (Ayllon et al. 2015, Barson et al. 2015, Czorlich et al. 2018, Pritchard et al. 2018, Aykanat et al. 2019). The so-called *vgll3* and *six6* genomic regions are named after the most prominent genes in their respective haploblocks on chromosomes 25 and 9, respectively. The *vgll3* genomic region on

chromosome 25 is associated with age at maturity (Ayllon et al. 2015, Barson et al. 2015), iteroparity (Aykanat et al. 2019), and possibly precocious male maturation in Atlantic salmon (Lepais et al. 2017). This genomic region also exhibits strong spatial divergence (Barson et al. 2015, Pritchard et al. 2018), and it has recently been shown to have been affected by natural selection over the last 36 years (equivalent to 4-6 salmon generations) in parallel to the changing age structure (Czorlich et al. 2018). The *six6* region on chromosome 9 is associated with sea age at maturity at the population level as a result of the strong correlation between the average allele frequency and average maturation age of populations (Barson et al. 2015). This region also exhibits the strongest signal of differentiation among European populations (Barson et al. 2015) and Tana/Teno River populations (Pritchard et al. 2018) and is hence distinguished as a critical genomic region for local adaptation. Genes found in these haploblocks appear to have a role in adipose or energy metabolism regulation in other organisms. The *vgll3* gene is an adipocyte inhibitor, the expression of which is correlated with body weight and gonadal adipose content in mice (Halperin et al. 2013). Recently, a strong selective sweep near the *vgll3* gene was postulated to be due to energy metabolism effects in humans in Mongolia (Nakayama et al. 2017). In turn, genes in the *six6* genomic region are involved in cell growth, cell differentiation, apoptosis in human cell lines (*PPM1A*, Lin et al. 2006), and myogenesis and skeletal muscle cell proliferation in zebrafish (*six1b*, Ridgeway and Skerjanc 2001, Bessarab et al. 2004, O'Brien et al. 2014) and act as an evolutionarily conserved regulator of eye development and the pituitary–hypothalamic axis (*six6*, Serikaku and Otousa 1994, Toy et al. 1998, Gallardo et al. 1999). Collectively, this suggests that the *vgll3* and *six6* haploblocks might have broad-scale roles in reproductive and life-history strategies in Atlantic salmon. However, how polymorphism in these regions may be translated to functional differences expressed in the wild is unclear.

Here, our objective was to test whether age-dependent differences in food acquisition efficiency are associated with the *vgll3* and *six6* genomic regions and discuss their role in explaining the genetic variation in age structure. We achieved this goal by assessing stomach content data from adult Atlantic salmon sampled along the coast during spawning migration and genotyping the same individuals for the *vgll3* and *six6* genomic regions using a targeted sequencing approach. Using a modelling framework that accounted for potentially confounding environmental and phenotypic variables, we tested whether variation in diet and resource acquisition strategies had a genetic component explained by the age at maturity-linked genomic regions. Elucidating the genetic interplay between age at maturity and diet breadth is crucial to better understand the dynamics and evolution of ecological

specialization and to better predict future demographic changes in Atlantic salmon populations under climate change.

Materials and Methods:

Sample collection

As part of a larger effort within the project "Sea salmon fishery, resource and potential (KOLARCTIC)", Atlantic salmon (*Salmo salar*), on their return migration to spawning grounds, were sampled and stomachs were collected between mid-May and late July in 2008 by local sea fishers with bend nets or bag nets along the Finnmark coast, northern Norway (Svenning et al. 2019, Figure 1). Sampled fish were measured (fork length, cm) and weighed (g); their sex and maturity were identified, and stomachs were frozen for later diet analysis. In addition, scales were sampled from all fish for age determination, categorization as wild or farmed fish according to ICES guidelines (ICES 2011, Svenning et al. 2019), and genetic analysis. The species composition of the diet was then characterized by morphologically identifying all prey items down to the species level and weighing them. Unidentified digested remains were also weighed. The identifiable portion of the diet in the dataset was overwhelmingly comprised of four fish species: sand eel (*Ammodytes* spp.), capelin (*Mallotus villosus*), herring (*Clupea harengus*), and haddock (*Melanogrammus aeglefinus*, see Results section for details). In the interest of analytical brevity, a few rare prey species were handled as follows: one gadoid fish was grouped with haddocks, both of which belong to the Gadiformes order, and negligible amounts of krill, other crustaceans, and Liparidae (0.2% of the total stomach weight) were categorized together with the unidentified material.

DNA extraction, microsatellite genotyping, and SNP genotyping by targeted sequencing

DNA was extracted from scales either using a QIAamp 96 DNA QIAcube HT Kit (Qiagen) following the manufacturer's protocol or according to (Elphinstone et al. 2003). Microsatellite genotyping of 31 markers was performed as outlined in (Ozerov et al. 2017). Samples were further genotyped by targeted sequencing at 173 SNP markers and the sex determination locus (sdy) as outlined in (Aykanat et al. 2016), with some modifications. Briefly, 174 genomic regions were first amplified in one multiplex PCR using locus-specific primers with truncated Illumina adapter sequences and using primer concentrations re-optimized for the Illumina platform (Supp. Table 1). The PCR products were

148 then treated with Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher) to
149 remove unused primers and nucleotides. After the treatment, the products were re-amplified with
150 adapter-specific primers containing Illumina and sample-specific dual-indexes. The index set was
151 optimized using the BARCOSEL software (Somervuo et al. 2018). The PCR products were then
152 pooled, purified and quantified with a Qubit 2.0 fluorimeter (Thermo Fisher) and analysed on a
153 fragment analyser (Agilent Technologies). The pooled library was then size selected using BluePippin
154 (Sage Sciences) to remove short unspecific products and checked on a fragment analyser. Finally,
155 samples were single-end sequenced using a 150-cycle high-output sequencing kit on a NextSeq 500
156 Illumina Sequencer following the manufacturer's guidelines. Loci with coverages over 12x were scored
157 as in (Aykanat et al. 2016). The two focal SNPs used in the analyses were *vgll3*$_{TOP}$, which exhibits the
158 strongest signal of association with age at maturity in the *vgll3* genomic region, and *six6*$_{TOP.LD}$ in the
159 *six6* haploblock on chromosome 9, the region that exhibited the second strongest association with sea
160 age at maturity prior to population structure correction and is 34.5 kb away from and in complete
161 linkage disequilibrium with the *six6*$_{TOP}$ SNP reported in Barson et al. (2015).

162 *Genetic stock identification (GSI)*

163 In total, 2023 samples that had greater than 80% success in regard to microsatellite genotyping were
164 assigned to their population of origin with 31 microsatellite markers as described in (Svenning et al.
165 2019) using the Bayesian GSI methodology described in (Pella and Masuda 2001) and implemented in
166 cBayes 5.0.1 (Neaves et al. 2005). In brief, the samples were allocated into 18 analysis groups, that is,
167 the combination of two time periods (May-June and July) and 10 fisheries regions, with each group
168 consisting of 30 to 288 samples for analysis. The GSI analyses were performed using five independent
169 chains of 100K iterations starting from three random stocks, and the last 10K iterations of each chain
170 were combined and used to assign individuals to their population of origin to remove the influence of
171 initial starting values. The baseline population data for the GSI analysis included genetic information
172 on 185 Atlantic salmon populations spanning from the Pechora River (Russia) in the east to the
173 Beiarelva River (Norway) in the west (see details in Ozerov et al. 2017). A total of 1372 (64.7%)
174 individuals were confidently assigned to a population of origin ($p > 0.7$). Individuals assigned to a
175 population with low confidence ($p < 0.7$, N=651, 30.7%) were kept in the dataset with the highest
176 ranked population assigned as the population of origin. Samples with no assignment due to low
177 genotyping success with microsatellites (N=82, 3.9%) were assigned a population of origin using

genotype information from the SNP panel. For that, an individual was assigned to the population to which it exhibited the highest genetic similarity, measured according to the average genetic similarity of focal individuals to the individuals in each population (as inferred with the GSI analysis in the previous step) using the *A.mat* function in the rrBLUP package (Endelman 2011) A very small subset individuals (N=16, < 1%) that also exhibited poor genotyping success with SNPs (less than 50 SNPs with successful genotyping) was randomly assigned to a population, in which population assignment probability is weighted over the total number of individuals that were assigned by GSI.

The final dataset contained 2121 individuals after excluding previously spawned and escaped farmed salmon. In the final dataset, 93.3% of the samples had visibly detectable developing gonads, confirming concordance between sea age and sea age at maturity. Missing data points for some variables were inferred from highly correlated variables. In that regard, missing sea age information (i.e., due to unclear formation of sea annulus for detecting the correct sea-age for some first time spawners) was inferred from length data for 15 (0.7%) individuals, where the likelihood of age, given the length, was substantially higher (>20 times) for the inferred age group than for other age groups. Additionally, for 21 (1.0%) individuals with missing length data, fit using coefficients of log(weight) to log(length) regression (adjusted $R^2 = 0.94$) was used to estimate the missing length information from the weight data. Finally, data for 33 (1.6%) individuals with missing $vgll3_{\mathrm{TOP}}$ genotype scores were inferred from the genotype score of an adjacent SNP marker in the genotyping panel, $vgll3_{\mathrm{Met54Thr}}$, which is in close physical proximity to $vgll3_{TOP}$ with high linkage ($r^2 = 0.79$).

Genetic and ecological basis of the diet scope

Unless otherwise noted, all statistical analyses were performed in R software v.3.2.5 (R Core Team, 2018). Either a two-component hurdle model (with binomial and the conditional negative binomial components) using the *glmmTMB* package (Brooks et al. 2017) or a binomial model (with a log link function) using the *gamm* function in the *mgcv* package (Wood 2011) was employed as the statistical model. In all models, population of origin was included as a random term to account for background population effects. To control for spatio-temporal variation, sampling location (longitude) and the day of sampling (Julian day, zero centred, and scaled to one standard deviation) were included as smoother terms. Longitude, which explained 90.7% of the spatial variation (i.e., sampling locations mostly occurred along a longitudinal axis (see Figure 1) and were included in the models as a surrogate for the two-dimensional spatial distribution to decrease the parametrization of the model. In addition to

including the genetic variation in the *six6* and *vgll3* genomic regions additively in the model (i.e., genotypes coded as a continuous factor with heterozygotes coded as the average of two homozygotes), age at maturity and residual length (log transformed total length after controlling for age at maturity) were also included in the model as categorical and continuous variables, respectively. All numeric variables were centred and scaled. For both genomic regions (*six6* and *vgll3*), alleles associated with late and early age at maturity were labelled as *L* and *E*, respectively.

The general model structure was as follows:

$$Z = SA + resL_{SA} + V_{SA} + S_{SA} + s(D) + s(L) + p + e$$

where Z is the vector of response variables given as a function of sea age (SA), scaled residual length and *vgll3* and *six6* genotypes nested within sea age ($resL_{SA}$, V_{SA} and $S_{SA,}$ respectively), the smoother functions of sampling day (D) and location (L), and normally distributed random variance due to the population (*p*) and individual (residual) effects. The genotypes are coded additively as 1, 2, and 3 (for *EE*, *EL* and *LL*, respectively). In the model, the scaled genotypes and residual length were analysed independently within sea age group (i.e., nested model), which provided a statistical framework suitable for testing hypotheses related to ontogenetic diet structure. In this model, a small number of 4SW individuals (N = 15, 0.7%, SW denotes number of winters salmon spent at sea prior to sampling.) was grouped and analysed within the 3SW for statistical coherence of the nested model. Models also accounted for spatio-temporal variation in the diet with smoothing spline functions. When using the *gamm* package, which provides a platform for generalized additive models, days and location were modelled with a smooth function (*s()*). When using *glmmTMB* package, which provides a platform for hurdle models but cannot directly model the smoother functions, an orthogonal spline design matrix with a low-rank thin-plate function was generated using the *spl* function in the *MCMCglmm* package (Hadfield 2010) in R and included in the model as fixed terms as a surrogate for the spatio-temporal spline functions. The number of knot points (*k*, which defines the curvature of the spline function) was set to five for both variables, but the results were robust to an increase in the *k* value, which did not qualitatively change the results (data not shown).

A number of variables pertaining to the diet content data were used as response variables in this study. Conceptually, these variables are linked to different aspects of diet acquisition mechanisms of this

236 species (e.g., Arrington et al. 2002) and may indicate different functional aspects associated with
237 performance and life history variation among individuals.

238 1) *The presence and amount of total diet content in the stomach:* We used a two-component hurdle
239 model to simultaneously account for stomach content quantity and empty stomach probability. By this,
240 we tested for the prevalence of the "feast and famine" diet acquisition strategy in Atlantic salmon as a
241 function of ontogeny and genotype, whereby large piscivorous fish species are predicted to experience
242 prolonged periods with empty stomachs in the interest of acquiring a high quantity of food (Arrington
243 et al. 2002, Armstrong and Schindler 2011). Both components in the hurdle model included the same
244 set of covariates (as described above). A logistic model with a log link was used to model the
245 probability of the presence of a prey item, whereas a zero truncated negative binomial model with a log
246 link was utilized as the conditional component. In this analysis, the total stomach weight, which had
247 excess zero elements and a right-tailed continuous distribution (ranging from 0.5 to 393.3 g), was
248 transformed to a discrete distribution by arbitrarily binning the total weight in 10 g increments, with
249 zero stomach content set as the first bin at a value of zero (Supp. Figure 1). This transformation
250 provided a distribution that can be modelled with the hurdle framework in the *glmmTMB* package
251 (Brooks et al. 2017).
252 2) *Total number and average weight of prey items in the stomach:* An increase in the total prey
253 weight in the stomach can be explained either by an increase in prey number or an increase in the
254 average prey weight. Therefore, we next investigated the contribution of these two components in
255 terms of explaining the model using the same statistical framework as above. Similar to the total prey
256 weight, the average prey item weight (ranging between 0.2 and 300.2 g) was also transformed to
257 discrete units by arbitrarily binning the data at 5 g intervals, with zero stomach content set as the first
258 bin (Supp. Figure 1).
259 3) *Relative prey composition:* Finally, to test whether sea age, size at age, or genotype is
260 associated with specific prey species, we modelled the prey composition, measured as the proportion of
261 a specific prey species contributing to the stomach content weight. We employed a binomial regression
262 using the *mgcv* package (Wood 2011) (as described above), whereby the proportion of a species in the
263 total prey weight was modelled as the response variable separately for all four species.

264 Extensively digested, unidentified content in the stomach (4.5% of the total stomach weight) was not
265 treated as diet material in order to accurately reflect the recent feeding activity (e.g., Jacobsen and

Hansen 2001). For all models, the effect size and confidence intervals were calculated with 10,000 parametric permutations of the model coefficients. To account for potential spurious inflation that may be caused by the inclusion of genetic data, the analytical pathway was repeated using 168 independent and putatively neutral markers that are present in the SNP panel, and focal SNPs were ranked across the background genetic effect (by comparing the genetic model and the null model at each SNP marker).

Results:

Out of the 2121 individuals examined in the final dataset, 992 individuals had identifiable prey items in their stomachs (46.8%). Four fish species, sand eel (*Ammodytes* spp.), capelin (*Mallotus villosus*), herring (*Clupea harengus*), and haddock (*Melanogrammus aeglefinus*), comprised the bulk of the diet content, representing 42.2 kg of the 44.2 kg quantified diet content (95.5%). In total, there were 2843 identifiable prey items in the datasets, with sand eel being the most abundant and herring being the largest percentage by weight (Supp. Figure 2). On average, prey size significantly differed among species, with haddock being the heaviest, followed by herring, capelin, and sand eel (Supp. Figure 2).

Prey probability and weight in the stomach as a function of sea age, size at age, and genetic variation

The two-component hurdle model revealed a striking negative relationship between the probability of non-empty stomach (e.g. presence of identifiable prey item in the stomach) and prey weight (grams) in the stomachs of Atlantic salmon as a function of sea age (Figure 2a, Supp. Table 2). Young age groups were more likely than older age groups to have non-empty stomachs. 1SW individuals were 1.45 (1.07 - 1.95, 90% CI, $p = 0.020$) and 2.39 (1.73 - 3.30, 90% CI, $p < 0.001$) times more likely to have any prey item in their stomachs than 2SW and 3SW individuals, respectively, and 2SW individuals were 1.65 times more likely to have a prey item in their stomachs than 3SW fish (1.35 - 2.00, 90% CI, $p < 0.001$). The decrease in non-empty stomach was significantly associated with residual size variation within the 2SW age group ($p = 0.027$, Figure 2b), with larger individuals having empty stomachs more often than the smaller-sized fish in the same age group.

The *six6*L* allele, which has a higher frequency in populations with an older sea age at maturity (Barson et al. 2015), was associated with an increase in the probability of non-empty stomach in an

294 age-dependent order, with a more pronounced effect in younger age groups (Figure 3a). Allelic
295 substitution from *E* to *L* in the *six6* genomic region (i.e. change in effect size by changing one allele of
296 the genotype) increased the probability of prey occurring in the stomach by 1.56 (1.06 – 2.30, 90% CI,
297 $p = 0.057$) and 1.25 times (1.08 - 1.45, 90% CI, $p < 0.014$) in the 1SW and 2SW groups, respectively
298 (Figure 3a). Both age groups exhibited significant or near significant age-dependent genotype effects
299 relative to the 3SW group ($p = 0.036$ and 0.051, respectively, Figure 3a). Strikingly, the increase in size
300 at age was in the opposite direction to the *six6*L* effect (Figures 2b & 3a) despite there being a
301 significant correlation between the two (Supp. Table 3), suggesting the occurrence of complex,
302 contrasting effects of *six6* across different phenotypic classes.

303 The conditional truncated negative binomial model suggested that young age groups had significantly
304 less prey content in their stomachs than older age groups despite a higher likelihood of having non-
305 empty stomach (Figure 2a). The contrasting results between the zero-inflated and truncated negative
306 binomial components suggest that resource acquisition strategies differ among age groups. The model
307 estimated, on average, 9.9 g (7.0 - 13.9, 90% CI), 24.8 g (21.8 – 28.6, 90% CI), and 41.5 g (33.8 – 50.9,
308 90% CI) of prey items in the stomachs of 1SW, 2SW and 3SW fish, respectively, all of which were
309 highly significantly different from one another ($p < 0.001$). Residual length at age also appears to be a
310 predictor of prey content, but only significantly so in the 2SW age group ($p = 0.027$, Figure 2b).

311 The conditional model suggested that the *six6*L* allele was associated with increased total stomach
312 weight in the young age groups (1SW and 2SW) but not in the 3SW group (Figure 3a). The allelic
313 substitution effect from *E* to *L* was significant and associated with a 1.87-fold (1.29-2.73, 90% CI, $p =$
314 0.006) increase in prey weight in the 1SW group and was marginal in the 2SW group, associated with a
315 1.14 (1.00-1.30, 90% CI, $p = 0.099$) increase in prey weight (Figure 3a).

316 The *vgll3* genomic region was not associated with diet content variation, suggesting no link between
317 the two (Figure 3b). However, the region may be subject to evolution via selection on sea age variation,
318 since the *vgll3* effect covaries with age at maturity and length at age. Accordingly, the effect of *vgll3*
319 was significant when these covarying phenotypes were not accounted for in the model (Supp. Table 4).
320 Spatio-temporal variance in the dataset was substantial in explaining diet variation in both components
321 of the hurdle model (Supp. Table 2, see also Supp. Figure 3). In general, diet presence and quantity
322 were the highest at the westerly end of the distribution, with a gradual decrease towards the east. At the

temporal scale, sampling days in the middle of the sampling period were associated with a higher presence and quantity of diet in the stomach (Supp. Figure 3).

Population of origin did not appear to be a significant source of diet variation and explained only a fraction of the total variation in diet content (Supp. Table 2). When the analysis was performed with samples assigned to a population of origin with high confidence (N=1372), the variance due to population was similarly small (e.g., Supp. Table 5 shows the results for total stomach weight as the response variable; the results for other response variables, prey size and prey weight, are not shown), and the model was less parsimonious than that without the population effect, as assessed by comparing the model fit by difference in their Akaike information criterion (ΔAIC = 3.42).

In our framework, digested, unidentified material in the stomach was not included in the analysis (e.g., Jacobsen and Hansen 2001). However, the results were qualitatively similar when digested material was included in the analysis (Supp. Table 6). A model including sex was less parsimonious and the term was not included as a parameter in the model (ΔAIC = 0.75). Finally, when the fit of the genetic models (*six6* and *vgll3*) was compared to the putatively neutral SNPs in the panel, *six6* ranked first out 167, confirming its significance, while *vgll3* was only ranked 123rd (Supp. Figure 4).

In general, both the number of prey items and the increase in the individual prey weight contributed to the variation in the total stomach weight (Figures 2 & 3, Supp. Tables 7 & 8). The 3SW age group was associated with significantly fewer prey items (0.49 prey items, 0.34-0.71, 90% CI) than the 1SW (0.99 prey items, 0.65-1.52, 90% CI, $p = 0.004$) and 2SW age groups (1.32 prey items, 1.01-1.71, 90% CI, $p < 0.001$), but the average prey weight was significantly heavier (27.40 g, 21.24-35.35, 90% CI) than that in the 1SW (2.68 g, 1.79-4.06, 90% CI, $p < 0.001$) and 2SW (7.83 g, 6.45-9.53, 90% CI, $p < 0.001$) salmon. The average prey weight, but not the prey number, was also significantly different between the 2SW and 1SW age groups ($p < 0.001$, Figure 2a). Size within age group also significantly influenced the number and size of prey. Larger fish within the 3SW age group had fewer ($p < 0.001$) but heavier ($p < 0.001$) prey items in the diet than smaller fish, and larger 2SW individuals consumed smaller prey items ($p < 0.001$, Figure 2b, Supp. Tables 7 & 8).

The number and size of prey items was also explained by the *six6* genotype in an age-dependent manner, with a more pronounced effect in the relatively young age groups. The *E* to *L* substitution in *six6* was associated with a 1.60-fold (1.08-2.36, 90% CI, $p = 0.048$) increase in prey number in the

1SW age group (Figure 3a). The allelic substitution was also associated with 1.65-fold (1.07-2.56, 90% CI, $p = 0.056$) and 1.22-fold (1.04-1.42, 90% CI, $p = 0.040$) increases in average prey weight in the 1SW and 2SW age groups, respectively, which was significant compared to that observed in the 3SW age group ($p = 0.018$ and 0.030, respectively, Figure 3a). Genetic variation in *vgll3* was not significantly associated with average individual prey weight or prey number after controlling for age at maturity (Figure 3b).

Relative prey composition as a function of sea age, size, and genetic variation

Finally, we modelled the relative prey composition, measured as the proportion of a specific prey species contributing to the stomach content weight. In general, prey composition substantially varied across different age groups, suggesting a change in prey composition as the fish grow older (Figure 4). In general, older age groups were more likely to prey on herring and haddock, while younger age groups preyed on capelin and sand eel. The same pattern was observed within age groups, (e.g. larger fish within an age group had proportionally more herring and haddock than smaller fish in the same age group) albeit generally not significantly (Supp. Figure 5), suggesting that size may be a contributing factor explaining prey composition. In all analyses, spatio-temporal variation was a significant component explaining the prey composition (Supp. Table 9).

Genetic variation in *six6* and *vgll3* did not appear to be a strong predictor of prey composition, but some notable associational trends existed for the two genomic regions (Supp. Figure 6, Supp. Table 9). Particularly, *E* to *L* substitution in *vgll3* is associated with 1.31 times (1.01-1.70, 90% CI, $p = 0.089$) and 1.49 times (1.03-2.16, 90% CI, $p = 0.076$) fewer capelin in the stomach relative to other prey species in the 2SW and 3SW age groups, respectively (Supp. Figure 6). Additionally, there was a significant age-dependent preference for capelin over herring associated with the *E* to *L* substitution in the *six6* genomic region (Supp. Figure 6). When compared to putatively neutral SNPs in the genotyping panel, capelin composition modelled with *vgll3* ranked 12th out of 164 SNPs (0.073, Supp. Figure 7), a value that is consistent with the analytically inferred p-value. Finally, when sea age and size at age were not controlled for, as expected, genetic variation in both the *vgll3* and *six6* genomic regions explained a substantial portion of the variation in relative prey composition, suggesting that genetic variation may further influence changes in prey composition via the association with size and age at maturity (Supp. Table 10).

Discussion:

In this study, we demonstrated that diet acquisition strategies in the sea vary with sea age in Atlantic salmon and that this variation is associated with genetic variation in key life history genomic regions, particularly in the *six6* genomic region. The variation in diet explained by sea age and size at age was mostly concordant, suggesting that size is the major driver of diet variation, influencing both the quantity and species composition of prey (Figures 2 & 4). Atlantic salmon prey on heavier but fewer prey as they grow older and larger, which seems to be a strategy that comes at the expense of a reduced prey acquisition probability (Figure 2a, e, Huey et al. 2001, Arrington et al. 2002). This pattern is consistent with the so-called "feast and famine" strategy observed among large piscivorous fish species (Arrington et al. 2002). The feast and famine feeding strategy is suggested to be an adaptation to maintain a positive energy balance at a large body size, especially when the acquisition of energy-rich food sources is unpredictable (Armstrong and Schindler 2011). Large Atlantic salmon appear to adopt this strategy, which is likely beneficial in terms of balancing the increase in energy costs associated with a large body size. A suite of physiological adaptations and metabolic adjustments, such as increased digestion capacity (Armstrong and Schindler 2011) and fat storage (Bustard 1967), may be associated with this strategy (Wang et al. 2006). For example, it has been shown that piscivorous species that adopt a feast and famine strategy maintain a large digestive tract, which provides quick food utilization when abundant prey are encountered (Armstrong and Schindler 2011). This physiological trade-off seems to be evolutionarily favourable for large fish when the prey distribution is stochastic despite the energetic costs of sustaining excess and energetically expensive digestive tissue (Armstrong and Schindler 2011). The feast and famine strategy in large Atlantic salmon may also be facilitated by other mechanistic processes, such as the trade-off of a lower success rate linked to larger prey or a lower attack rate associated with increasing size. Nonetheless, variation in foraging strategies among different age groups results in a large diet breadth, efficient resource partitioning, and reduced intraspecific competition among age groups, which subsequently promotes their co-existence (e.g., Polis 1984, Smith and Skulason 1996, Svanback and Bolnick 2007). It is unclear what physiological or behavioural modifications are associated with the differential feeding strategies among the various age groups in Atlantic salmon. Nonetheless, changes in marine food webs may alter the density and composition of prey available to different age groups and hence alter the age-dependent selection landscape, potentially leading to adaptive changes in age structure. Our results confirm the value of

411 Atlantic salmon as a model species to study the evolutionary physiology of starvation and feeding in
412 response to environmental changes in the wild.

413 Although salmon are generally considered to be diet generalists, the association between life history
414 genomic regions (*six6* and marginally *vgll3*) and diet acquisition suggests that genetically controlled
415 intraspecific diet specialization occurs in Atlantic salmon. This genetic variation may be linked to
416 specialized dietary adaptations (e.g., physiological, morphological or behavioural) allowing the
417 efficient utilization of diverse diet sources, resulting in increased niche breadth and reduced
418 intraspecific competition (Dalmo et al. 1997, Bolnick et al. 2003, Bolnick and Fitzpatrick 2007,
419 Svanback and Bolnick 2007). In particular, the *six6*L* allele was associated with increased content in
420 the stomach, especially in young age groups, which was explained by both an increase in the number of
421 prey items and an increase in the average prey weight in the stomach. Intriguingly, however, the *six6*L*
422 allele was associated with a larger fish size within all sea ages (Supp. Table 3, see also, Barson et al.
423 2015). Given that larger size at a given sea age is indicative of higher performance and fitness in adult
424 salmon (e.g., Fleming 1996), the *six6*L* allele (being linked to larger size) is predicted to have a
425 selective advantage over the *E* allele and is hence predicted to prevail within and among populations. In
426 contrast, the *six6* genomic region is highly variable within and among populations (Barson et al. 2015,
427 Pritchard et al. 2018), and balancing selection appears to be the pervasive mode of evolution (Barson et
428 al. 2015), with both alleles exerting some fitness advantage. This apparent contradiction may be
429 explained by changes in the fitness landscape during the life cycle of an individual (i.e., fitness trade-
430 offs within the lifetime of an individual associated with the *six6* region) or across generations. For
431 example, genetic variation in *six6* may be under balancing selection via antagonistic genetic
432 correlations in diet acquisition over the life cycle. Indeed, some results indicate that individuals having
433 high freshwater growth may show poor seawater growth (Einum et al. 2002), prescribing further
434 hypothesis linking it to genetic variation in *six6* genomic region.

435 Although our results suggest superior performance of the *six6*L* allele at the adult stage, the opposite
436 may be true at earlier life stages, when the prey species composition is substantially different, with
437 significantly more invertebrates in the diet (Jacobsen and Hansen 2001, Rikardsen et al. 2004,
438 Haugland et al. 2006), and different ecological drivers affect performance (Mittelbach et al. 2014,
439 Sanchez-Hernandez et al. 2019). For example, if increased metabolic costs are linked with increased
440 prey content in the stomach, a trait associated with *six6*L*, this may not be an optimal acquisition

441 strategy in younger years (i.e. juveniles prior to, or in the early phases of marine migration) when the
442 energy density of available prey cannot compensate for the greater effort (McNamara and Houston
443 1996, Enberg et al. 2012). Such antagonistic genetic correlations between early- and late-life histories
444 in diet acquisition may maintain polymorphism in the region. Alternatively, year-to-year variation in
445 population-specific (e.g., density dependent) or ecosystem-level processes (i.e., prey composition,
446 food-web dynamics) may alter the adaptive landscape of diet acquisition (Smith and Skulason 1996),
447 resulting in fluctuating selection and a change in the direction of selection among alleles, which would
448 maintain the genetic variation in the region. Indeed, in the year 2008, returning salmon had a notably
449 older sea age at maturity in northern Norway than that observed in more recent years (i.e., the oldest in
450 the last 20 years, see (Anon 2014). This is consistent with observed patterns that *six6*L* is linked with
451 larger size, perhaps as a result of providing a selective advantage for that particular year class.
452 However, more research is required to explicitly test this possibility. The *six6* genomic region is highly
453 spatially differentiated among populations (Barson et al. 2015, Pritchard et al. 2018). This suggests that
454 any selection acting on *six6* as a result of selection on diet acquisition would influence the fitness of
455 populations differentially, correlated with their average allele frequency. Hence, genetic variation in
456 *six6* may be linked to the differential survival of populations at sea and should be closely monitored in
457 population management.

458 The genetic variation in the v*gll3* genomic region had no clear effect on diet quantity, but there was a
459 marginal, albeit consistent, association with higher capelin composition in the stomach (Supp. Figures
460 6 & 7). Capelin is a key component in the Barents Sea ecosystem with critical bottom-up influence on
461 the dynamics of many marine organisms, including cod, birds, and sea mammals (Gjøsæter et al. 2016).
462 Capelin abundances have fluctuated considerably over recent decades, with some critical collapses
463 mainly induced by occasional juvenile herring fluxes to the Barents system (Gjøsæter et al. 2016).
464 Capelin schools occupy feeding grounds in the central and northern Barents Sea, and they migrate to
465 the coastal regions for spawning (Gjøsæter et al. 2016), the time at which salmon post-smolts and
466 returning adults utilize them as a food resource (Rikardsen and Dempson 2010, this study). Individuals
467 with the *vgll3*L* allele, which is associated with a later sea-age at maturity (Barson et al. 2015), were
468 marginally less likely to feed on capelin, particularly in older age groups (2SW and 3SW). In addition,
469 in contrast to herring, which was the most common prey item in the stomachs of the individuals in the
470 2SW and 3SW age groups (Figure 4), capelin is likely a lower-energy prey item for Atlantic salmon
471 (Elliott and Gaston 2008, Hedeholm et al. 2011, Renkawitz et al. 2015). This supports the notion that

*vgll3*L* may contribute to foraging adaptations to support the high-energy demands associated with the larger body size of late maturing fish. However, the mechanisms driving such a compositional difference have yet to be determined.

Overall, genetic variation in both life history genomic regions appears to have a role in intraspecific diet specialization, but the mechanisms remain to be clarified. Although the underlying mechanism of diet specialization is complex (e.g., Mittelbach et al. 2014) and challenging to disentangle, performance trade-offs across different ecological settings and the life cycle are likely driving the life-history variation associated with these genomic regions. Analyses of datasets collected in the wild may suffer from confounding effects that co-vary with both the response variable and parameters of interest and generate spurious associations if they are not accounted for. In this study, a highly controlled linear model was employed to account for environmental and intrinsic parameters with potential confounding effects. First, resource availability at sea, e.g., the prey species density and distribution, is highly variable across time and space, even at small scales. Likewise, during their return migration, Atlantic salmon may be non-randomly distributed in relation to their life history, genotype, and population of origin (Svenning et al. 2019). For example, relatively early run timing is linked to both later maturation (Jonsson and Jonsson 2011) and *six6*L* (Cauwelier et al. 2018, Pritchard et al. 2018), a pattern that concordantly holds in our dataset (Supp. Table 11). Hence, the analytical framework controlled for spatio-temporal variation and also accounted for non-linear changes through space and time (Supp. Figure 3). Similarly, sea age at maturity and size within an age group exhibited strong links with both diet and genetic variation in the life history genomic regions. By accounting for these phenotypes in the model, we were able to exclude the possibility of modelling the genetic variation via the effects of these intermediate phenotypes. Therefore, our framework was rather robust to drawbacks related to confounding factors observed in wild settings. Finally, variation in diet due to the population of origin was also accounted for in the model as a random intercept but did not explain significant diet variation at sea (Supp. Tables 2 & 5).

Quantifying resource acquisition via stomach content analysis is an integral component of trophic studies in ecology and evolution. In this study, we used stomach content information from a single time point as a proxy for diet acquisition. The analysis of diet content may be difficult for a number of reasons, such as the challenges in accounting for the diverse nature of diet content data, high percentage of digested items, and the diversity of metrics for statistical analysis (e.g., Rice 1988, Cortés

1997, de Crespin de Billy 2000, Baker et al. 2014). On the other hand, the relatively small number of diet species in our dataset, all of which were fishes, and the small proportion of undigested material provided us with a robust quantification of the diet and a powerful statistical framework with ecologically relevant response variables. Finally, the three most common prey species in our dataset (e.g., herring, capelin and sand eel) are from similar trophic levels independent of size or ontogeny (Dommasnes et al. , Bentley et al. 2017). Therefore, alternative approaches such as stable isotope analysis, which is sensitive to diet variation only across trophic levels, would have been unable to tease the observed variation apart, suggesting that stomach content analysis is a more robust approach when diet composition is variable only within similar trophic levels.

Marine ecosystems, which are composed of mostly poikilothermic species, are sensitive and highly responsive to temperature-driven changes (e.g., Clarke 2003, Sydeman et al. 2015). The Arctic region is particularly sensitive to global climate change (Polyakov et al. 2010), with significant anthropogenic effects further shaping the marine food webs in the region. Over the last 40 years, the abundance of Atlantic salmon has been declining, and the age structure has been shifting towards a younger age at maturity (Chaput 2012, Czorlich et al. 2018, Erkinaro et al. 2018). Such changes in demography are likely the result of bottom-up changes in prey community structure, likely fuelled by climate-induced changes to the ecosystem (Frederiksen et al. 2006, Todd et al. 2008). In this study, we demonstrated that the inter-individual variation in diet specialization is linked with age structure as well as the genetic variation in *six6* and *vgll3*, two genomic regions with substantial influence on life history variation and population divergence. This heritable intraspecific variation in diet specialization likely plays an important role in salmon life history by both promoting the niche breadth of species and enabling evolutionary responses in populations to changes in food composition. Given that both genomic regions are highly differentiated among populations, evolutionary response and the resulting demographic trajectories likely differ among populations. Future work should focus on characterizing the underlying physiological and/or behavioural mechanisms linking genetic variation with salmon diet acquisition to better predict the evolutionary response of populations to changing environments.

Acknowledgments

We acknowledge the Atlantic Sea salmon fishing organisations in Finnmark county, and especially fishers who collected more than 2000 stomachs from wild Atlantic salmon used in this study. Ursula Lönnqvist is thanked for laboratory assistance. This project received funding from the Academy of

Finland (project numbers 318939 to TA, 307593, 302873 to CRP), from the European Union, Kolarctic ENPI CBC project "Trilateral cooperation on our common resource; the Atlantic salmon in the Barents region (KO197)" (https://prosjekt.fylkesmannen.no/Kolarcticsalmon), and The Research Council of Norway project no. 244086/E50. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References:

Anon. 2014. Status of the River Tana salmon populations. Report of the working group on salmon monitoring and research in the Tana River system.

Armstrong, J. B., and D. E. Schindler. 2011. Excess digestive capacity in predators reflects a life of feast and famine. Nature **476**:84-87.

Arrington, D. A., K. O. Winemiller, W. F. Loftus, and S. Akin. 2002. How often do fishes "run on empty"? Ecology **83**:2145-2151.

Aykanat, T., M. Lindqvist, V. L. Pritchard, and C. R. Primmer. 2016. From population genomics to conservation and management: a workflow for targeted analysis of markers identified using genome-wide approaches in Atlantic salmon Salmo salar. Journal of Fish Biology **89**:2658-2679.

Aykanat, T., M. Ozerov, J. P. Vaha, P. Orell, E. Niemela, J. Erkinaro, and C. R. Primmer. 2019. Co-inheritance of sea age at maturity and iteroparity in the Atlantic salmon *vgll3* genomic region. Journal of Evolutionary Biology.

Ayllon, F., E. Kjaerner-Semb, T. Furmanek, V. Wennevik, M. F. Solberg, G. Dahle, G. L. Taranger, K. A. Glover, M. S. Almen, C. J. Rubin, R. B. Edvardsen, and A. Wargelius. 2015. The *vgll3* Locus Controls Age at Maturity in Wild and Domesticated Atlantic Salmon (Salmo salar L.) Males. PLoS Genetics **11**:e1005628.

Baker, R., A. Buckland, and M. Sheaves. 2014. Fish gut content analysis: robust measures of diet composition. Fish and Fisheries **15**:170-177.

Barson, N. J., T. Aykanat, K. Hindar, M. Baranski, G. H. Bolstad, P. Fiske, C. Jacq, A. J. Jensen, S. E. Johnston, S. Karlsson, M. Kent, T. Moen, E. Niemela, T. Nome, T. F. Naesje, P. Orell, A. Romakkaniemi, H. Saegrov, K. Urdal, J. Erkinaro, S. Lien, and C. R. Primmer. 2015. Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature **528**:405-408.

Bentley, J. W., N. Serpetti, and J. J. Heymans. 2017. Investigating the potential impacts of ocean warming on the Norwegian and Barents Seas ecosystem using a time-dynamic food-web model. Ecological Modelling **360**:94-107.

Bessarab, D. A., S. W. Chong, and V. Korzh. 2004. Expression of zebrafish *six1* during sensory organ development and myogenesis. Developmental Dynamics **230**:781-786.

Bolnick, D. I., P. Amarasekare, M. S. Araujo, R. Burger, J. M. Levine, M. Novak, V. H. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution **26**:183-192.

Bolnick, D. I., and M. S. Araujo. 2011. Partitioning the relative fitness effects of diet and trophic morphology in the threespine stickleback. Evolutionary Ecology Research **13**:439-459.

Bolnick, D. I., and B. M. Fitzpatrick. 2007. Sympatric Speciation: Models and Empirical Evidence. Annual Review of Ecology, Evolution, and Systematics **38**:459-487.

Bolnick, D. I., R. Svanback, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, and M. L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. American Naturalist **161**:1-28.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Machler, and B. M. Bolker. 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. R Journal **9**:378-400.

Bustard, H. R. 1967. Gekkonid lizards adapt fat storage to desert environments. Science **158**:1197-1198.

Carter, A. J., A. W. Goldizen, and S. A. Tromp. 2010. Agamas exhibit behavioral syndromes: bolder males bask and feed more but may suffer higher predation. Behavioral Ecology **21**:655-661.

578 Cauwelier, E., J. Gilbey, J. Sampayo, L. Stradmeyer, and S. J. Middlemas. 2018. Identification of a single genomic region
579 associated with seasonal river return timing in adult Scottish Atlantic salmon (Salmo salar), using a genome-wide
580 association study. Canadian Journal of Fisheries and Aquatic Sciences **75**:1427-1435.
581 Chaput, G. 2012. Overview of the status of Atlantic salmon (*Salmo salar*) in the North Atlantic and trends in marine
582 mortality. ICES Journal of Marine Science **69**:1538-1548.
583 Claessen, D., and U. Dieckmann. 2002. Ontogenetic niche shifts and evolutionary branching in size-structured populations.
584 Evolutionary Ecology Research **4**:189-217.
585 Clarke, A. 2003. Costs and consequences of evolutionary temperature adaptation. Trends in Ecology & Evolution **18**:573-
586 581.
587 Cortés, E. 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: application to
588 elasmobranch fishes. Canadian Journal of Fisheries and Aquatic Sciences **54**:726-738.
589 Czorlich, Y., T. Aykanat, J. Erkinaro, P. Orell, and C. R. Primmer. 2018. Rapid sex-specific evolution of age at maturity is
590 shaped by genetic architecture in Atlantic salmon. Nat Ecol Evol **2**:1800-1807.
591 Dalmo, R. A., K. Ingebrigtsen, and J. Bogwald. 1997. Non-specific defence mechanisms in fish, with particular reference to
592 the reticuloendothelial system (RES). Journal of Fish Diseases **20**:241-273.
593 Daufresne, M., K. Lengfellner, and U. Sommer. 2009. Global warming benefits the small in aquatic ecosystems. Proc Natl
594 Acad Sci U S A **106**:12788-12793.
595 de Crespin de Billy, V. 2000. Biplot presentation of diet composition data: an alternative for fish stomach contents analysis.
596 Journal of Fish Biology **56**:961-973.
597 Devictor, V., J. Clavel, R. Julliard, S. Lavergne, D. Mouillot, W. Thuiller, P. Venail, S. Villéger, and N. Mouquet. 2010.
598 Defining and measuring ecological specialization. Journal of Applied Ecology **47**:15-25.
599 Dommasnes, A., V. Christensen, B. Ellertsen, C. Kvamme, W. Melle, L. Nøttestad, P. Torstein, S. Tjelmeland, and D.
600 Zeller. An ecopath model for the Norwegian sea and Barents sea.
601 Einum, S., E. B. Thorstad, and T. F. Naesje. 2002. Growth rate correlations across life-stages in female Atlantic salmon.
602 Journal of Fish Biology **60**:780-784.
603 Elliott, K. H., and A. J. Gaston. 2008. Mass-length relationships and energy content of fishes and invertebrates delivered to
604 nestling Thick-billed Murres *Uria lomvia* in the Canadian Arctic, 1981–2007. Marine Ornithology **36**:25–34.
605 Elmer, K. R., T. K. Lehtonen, A. F. Kautt, C. Harrod, and A. Meyer. 2010. Rapid sympatric ecological differentiation of
606 crater lake cichlid fishes within historic times. BMC Biology **8**:60.
607 Elphinstone, M. S., G. N. Hinten, M. J. Anderson, and C. J. Nock. 2003. An inexpensive and high-throughput procedure to
608 extract and purify total genomic DNA for population studies. Molecular Ecology Notes **3**:317-320.
609 Enberg, K., C. Jørgensen, E. S. Dunlop, Ø. Varpe, D. S. Boukal, L. Baulier, S. Eliassen, and M. Heino. 2012. Fishing-
610 induced evolution of growth: concepts, mechanisms and the empirical evidence. Marine Ecology **33**:1-25.
611 Endelman, J. B. 2011. Ridge Regression and Other Kernels for Genomic Selection with R Package rrBLUP. Plant Genome
612 **4**:250-255.
613 Engen, S., and N. C. Stenseth. 1989. Age-specific optimal diets and optimal foraging tactics: a life-historic approach.
614 Theoretical Population Biology **36**:281-295.
615 Erkinaro, J., Y. Czorlich, P. Orell, J. Kuusela, M. Falkegård, M. Länsman, H. Pulkkinen, C. R. Primmer, and E. Niemelä.
616 2018. Life history variation across four decades in a diverse population complex of Atlantic salmon in a large
617 subarctic river. Canadian Journal of Fisheries and Aquatic Sciences.
618 Erkinaro, J., J. B. Dempson, M. Julkunen, and E. Niemelä. 1997. Importance of ontogenetic habitat shifts to juvenile output
619 and life history of Atlantic salmon in a large subarctic river: an approach based on analysis of scale characteristics.
620 Journal of Fish Biology **51**:1174-1185.
621 Fleming, I. A. 1996. Reproductive strategies of Atlantic salmon: ecology and evolution. Reviews in Fish Biology and
622 Fisheries **6**:379-416.
623 Fox, L. R., and P. A. Morrow. 1981. Specialization: species property or local phenomenon? Science **211**:887-893.
624 Frederiksen, M., M. Edwards, A. J. Richardson, N. C. Halliday, and S. Wanless. 2006. From plankton to top predators:
625 bottom-up control of a marine food web across four trophic levels. Journal of Animal Ecology **75**:1259-1268.
626 Friedland, K. D., and R. E. Haas. 1996. Marine post-smolt growth and age at maturity of Atlantic salmon. Journal of Fish
627 Biology **48**:1-15.
628 Gallardo, M. E., J. Lopez-Rios, I. Fernaud-Espinosa, B. Granadino, R. Sanz, C. Ramos, C. Ayuso, M. J. Seller, H. G.
629 Brunner, P. Bovolenta, and S. Rodriguez de Cordoba. 1999. Genomic cloning and characterization of the human
630 homeobox gene *SIX6* reveals a cluster of *SIX* genes in chromosome 14 and associates *SIX6* hemizygosity with
631 bilateral anophthalmia and pituitary anomalies. Genomics **61**:82-91.

Gjøsæter, H., E. H. Hallfredsson, N. Mikkelsen, B. Bogstad, and T. Pedersen. 2016. Predation on early life stages is decisive for year-class strength in the Barents Sea capelin (*Mallotus villosus*) stock. ICES Journal of Marine Science: Journal du Conseil **73**:182-195.

Gotthard, K. 2000. Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. Journal of Animal Ecology **69**:896-902.

Hadfield, J. D. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software **33**:1-22.

Halperin, D. S., C. Pan, A. J. Lusis, and P. Tontonoz. 2013. Vestigial-like 3 is an inhibitor of adipocyte differentiation. Journal of Lipid Research **54**:473-481.

Haugland, M., J. Holst, M. Holm, and L. Hansen. 2006. Feeding of Atlantic salmon (*Salmo salar* L.) post-smolts in the Northeast Atlantic. ICES Journal of Marine Science **63**:1488-1500.

Hedeholm, R., P. Grønkjær, and S. Rysgaard. 2011. Energy content and fecundity of capelin (*Mallotus villosus*) along a 1,500-km latitudinal gradient. Marine Biology **158**:1319-1330.

Heino, M., and V. Kaitala. 1999. Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. Journal of Evolutionary Biology **12**:423-429.

Huey, R. B., E. R. Pianka, and L. J. Vitt. 2001. How often do lizards "run on empty"? Ecology **82**:1-7.

Hvidsten, N. A., A. J. Jensen, A. H. Rikardsen, B. Finstad, J. Aure, S. Stefansson, P. Fiske, and B. O. Johnsen. 2009. Influence of sea temperature and initial marine feeding on survival of Atlantic salmon *Salmo salar* post-smolts from the Rivers Orkla and Hals, Norway. Journal of Fish Biology **74**:1532-1548.

ICES. 2011. Report of the Workshop on Age Determination of Salmon (WKADS). Galway, Ireland.

Jacobsen, J. A., and L. P. Hansen. 2001. Feeding habits of wild and escaped farmed Atlantic salmon, *Salmo salar* L., in the Northeast Atlantic. ICES Journal of Marine Science **58**:916-933.

Jensen, H., M. Kiljunen, and P. A. Amundsen. 2012. Dietary ontogeny and niche shift to piscivory in lacustrine brown trout Salmo trutta revealed by stomach content and stable isotope analyses. Journal of Fish Biology **80**:2448-2462.

Jonsson, B., and N. Jonsson. 2011. Ecology of Atlantic salmon and brown trout : habitat as a template for life histories. Springer, Dordrecht.

Jonsson, N., B. Jonsson, and L. P. Hansen. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo salar*. Journal of Animal Ecology **66**:425-436.

Lepais, O., A. Manicki, S. Glise, M. Buoro, and A. Bardonnet. 2017. Genetic architecture of threshold reaction norms for male alternative reproductive tactics in Atlantic salmon (Salmo salar L.). Sci Rep **7**:43552.

Lin, X., X. Duan, Y. Y. Liang, Y. Su, K. H. Wrighton, J. Long, M. Hu, C. M. Davis, J. Wang, F. C. Brunicardi, Y. Shi, Y. G. Chen, A. Meng, and X. H. Feng. 2006. PPM1A functions as a Smad phosphatase to terminate TGFbeta signaling. Cell **125**:915-928.

Machovsky-Capuska, G. E., A. M. Senior, S. J. Simpson, and D. Raubenheimer. 2016. The Multidimensional Nutritional Niche. Trends in Ecology & Evolution **31**:355-365.

MacKenzie, K. M., C. N. Trueman, M. R. Palmer, A. Moore, A. T. Ibbotson, W. R. C. Beaumont, and I. C. Davidson. 2012. Stable isotopes reveal age-dependent trophic level and spatial segregation during adult marine feeding in populations of salmon. ICES Journal of Marine Science **69**:1637-1645.

McNamara, J. M., and A. I. Houston. 1996. State-dependent life histories. Nature **380**:215-221.

Mittelbach, G. G., N. G. Ballew, M. K. Kjelvik, and D. Fraser. 2014. Fish behavioral types and their ecological consequences. Canadian Journal of Fisheries and Aquatic Sciences **71**:927-944.

Mittelbach, G. G., and L. Persson. 1998. The ontogeny of piscivory and its ecological consequences. Canadian Journal of Fisheries and Aquatic Sciences **55**:1454-1465.

Mousseau, T. A., B. Sinervo, and J. A. Endler. 2000. Adaptive genetic variation in the wild. Oxford University Press, New York.

Nakayama, K., J. Ohashi, K. Watanabe, L. Munkhtulga, and S. Iwamoto. 2017. Evidence for Very Recent Positive Selection in Mongolians. Molecular Biology and Evolution.

Neaves, P. I., C. G. Wallace, J. R. Candy, and T. D. Beacham. 2005. CBayes: computer program for mixed-stock analysis of allelic data, version v5.01.

O'Brien, J. H., L. Hernandez-Lagunas, K. B. Artinger, and H. L. Ford. 2014. MicroRNA-30a regulates zebrafish myogenesis through targeting the transcription factor Six1. Journal of Cell Science **127**:2291-2301.

Ozerov, M., J.-P. Vähä, V. Wennevik, E. Niemelä, M.-A. Svenning, S. Prusov, R. Diaz Fernandez, L. Unneland, A. Vasemägi, M. Falkegård, T. Kalske, and B. Christiansen. 2017. Comprehensive microsatellite baseline for genetic stock identification of Atlantic salmon (*Salmo salar* L.) in northernmost Europe. ICES Journal of Marine Science.

Pella, J., and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fishery Bulletin **99**:151-167.

688 Pershing, A. J., M. A. Alexander, C. M. Hernandez, L. A. Kerr, A. Le Bris, K. E. Mills, J. A. Nye, N. R. Record, H. A.
689 Scannell, J. D. Scott, G. D. Sherwood, and A. C. Thomas. 2015. Slow adaptation in the face of rapid warming
690 leads to collapse of the Gulf of Maine cod fishery. Science **350**:809-812.
691 Polis, G. A. 1984. Age Structure Component of Niche Width and Intraspecific Resource Partitioning - Can Age-Groups
692 Function as Ecological Species. American Naturalist **123**:541-564.
693 Polyakov, I. V., L. A. Timokhov, V. A. Alexeev, S. Bacon, I. A. Dmitrenko, L. Fortier, I. E. Frolov, J.-C. Gascard, E.
694 Hansen, V. V. Ivanov, S. Laxon, C. Mauritzen, D. Perovich, K. Shimada, H. L. Simmons, V. T. Sokolov, M.
695 Steele, and J. Toole. 2010. Arctic Ocean Warming Contributes to Reduced Polar Ice Cap. Journal of Physical
696 Oceanography **40**:2743-2756.
697 Pritchard, V. L., H. Makinen, J. P. Vaha, J. Erkinaro, P. Orell, and C. R. Primmer. 2018. Genomic signatures of fine-scale
698 local selection in Atlantic salmon suggest involvement of sexual maturation, energy homeostasis and immune
699 defence-related genes. Molecular Ecology **27**:2560-2575.
700 Renkawitz, M. D., T. F. Sheehan, H. J. Dixon, and R. Nygaard. 2015. Changing trophic structure and energy dynamics in
701 the Northwest Atlantic: implications for Atlantic salmon feeding at West Greenland. Marine Ecology Progress
702 Series **538**:197-211.
703 Reznick, D. 2016. Hard and Soft Selection Revisited: How Evolution by Natural Selection Works in the Real World.
704 Journal of Heredity **107**:3-14.
705 Reznick, D., and J. A. Endler. 1982. The Impact of Predation on Life History Evolution in Trinidadian Guppies (Poecilia
706 Reticulata). Evolution **36**:160-177.
707 Reznick, D. N., and C. K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies
708 reveal about the conditions that promote adaptive evolution. Genetica **112**:183-198.
709 Rice, J. C. 1988. Repeated cluster analysis of stomach contents data: method and application to diet of cod in NAFO
710 division 3L. Environmental Biology of Fishes **21**:263-277.
711 Ridgeway, A. G., and I. S. Skerjanc. 2001. *Pax3* is essential for skeletal myogenesis and the expression of *Six1* and *Eya2*.
712 Journal of Biological Chemistry **276**:19033-19039.
713 Rikardsen, A. H., and J. B. Dempson. 2010. Dietary Life-Support: The Food and Feeding of Atlantic Salmon at Sea. Pages
714 115-143 Atlantic Salmon Ecology. Wiley-Blackwell.
715 Rikardsen, A. H., M. Haugland, P. A. Bjorn, B. Finstad, R. Knudsen, J. B. Dempson, J. C. Holst, N. A. Hvidsten, and M.
716 Holm. 2004. Geographical differences in marine feeding of Atlantic salmon post-smolts in Norwegian fjords.
717 Journal of Fish Biology **64**:1655-1679.
718 Sanchez-Hernandez, J., A. D. Nunn, C. E. Adams, and P. A. Amundsen. 2019. Causes and consequences of ontogenetic
719 dietary shifts: a global synthesis using fish models. Biological Reviews of the Cambridge Philosophical Society
720 **94**:539-554.
721 Serikaku, M. A., and J. E. Otousa. 1994. Sine Oculis Is a Homeobox Gene Required for Drosophila Visual-System
722 Development. Genetics **138**:1137-1150.
723 Sexton, J. P., J. Montiel, J. E. Shay, M. R. Stephens, and R. A. Slatyer. 2017. Evolution of Ecological Niche Breadth.
724 Annual Review of Ecology, Evolution, and Systematics **48**:183-206.
725 Smith, T. B., and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds.
726 Annual Review of Ecology and Systematics **27**:111-133.
727 Somervuo, P., P. Koskinen, P. Mei, L. Holm, P. Auvinen, and L. Paulin. 2018. BARCOSEL: a tool for selecting an optimal
728 barcode set for high-throughput sequencing. BMC Bioinformatics **19**:257.
729 Svanback, R., and D. I. Bolnick. 2007. Intraspecific competition drives increased resource use diversity within a natural
730 population. Proc Biol Sci **274**:839-844.
731 Svanback, R., and L. Persson. 2004. Individual diet specialization, niche width and population dynamics: implications for
732 trophic polymorphisms. Journal of Animal Ecology **73**:973-982.
733 Svenning, M.-A., M. Falkegård, E. Niemelä, J.-P. Vähä, V. Wennevik, M. Ozerov, S. Prusov, J. B. Dempson, M. Power, P.
734 Fauchald, and D. Gomez-Uchida. 2019. Coastal migration patterns of the four largest Barents Sea Atlantic salmon
735 stocks inferred using genetic stock identification methods. ICES Journal of Marine Science.
736 Sydeman, W. J., E. Poloczanska, T. E. Reed, and S. A. Thompson. 2015. Climate change and marine vertebrates. Science
737 **350**:772-777.
738 Taranger, G. L., M. Carrillo, R. W. Schulz, P. Fontaine, S. Zanuy, A. Felip, F. A. Weltzien, S. Dufour, O. Karlsen, B.
739 Norberg, E. Andersson, and T. Hansen. 2010. Control of puberty in farmed fish. General and Comparative
740 Endocrinology **165**:483-515.
741 Thorpe, J. E., M. Mangel, N. B. Metcalfe, and F. A. Huntingford. 1998. Modelling the proximate basis of salmonid life-
742 history variation, with application to Atlantic salmon, *Salmo salar* L. Evolutionary Ecology **12**:581-599.

743 Todd, C. D., S. L. Hughes, C. T. Marshall, J. C. MacLean, M. E. Lonergan, and E. M. Biuw. 2008. Detrimental effects of
744 recent ocean surface warming on growth condition of Atlantic salmon. Global Change Biology **14**:958-970.
745 Toy, J., J. M. Yang, G. S. Leppert, and O. H. Sundin. 1998. The *Optx2* homeobox gene is expressed in early precursors of
746 the eye and activates retina-specific genes. Proc Natl Acad Sci U S A **95**:10643-10648.
747 Wang, T., C. C. Hung, and D. J. Randall. 2006. The comparative physiology of food deprivation: from feast to famine.
748 Annual Review of Physiology **68**:223-251.
749 Werner, E. E., and J. F. Gilliam. 1984. The Ontogenetic Niche and Species Interactions in Size-Structured Populations.
750 Annual Review of Ecology and Systematics **15**:393-425.
751 Wood, S. N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric
752 generalized linear models. Journal of the Royal Statistical Society Series B-Statistical Methodology **73**:3-36.

753

754

Figures:

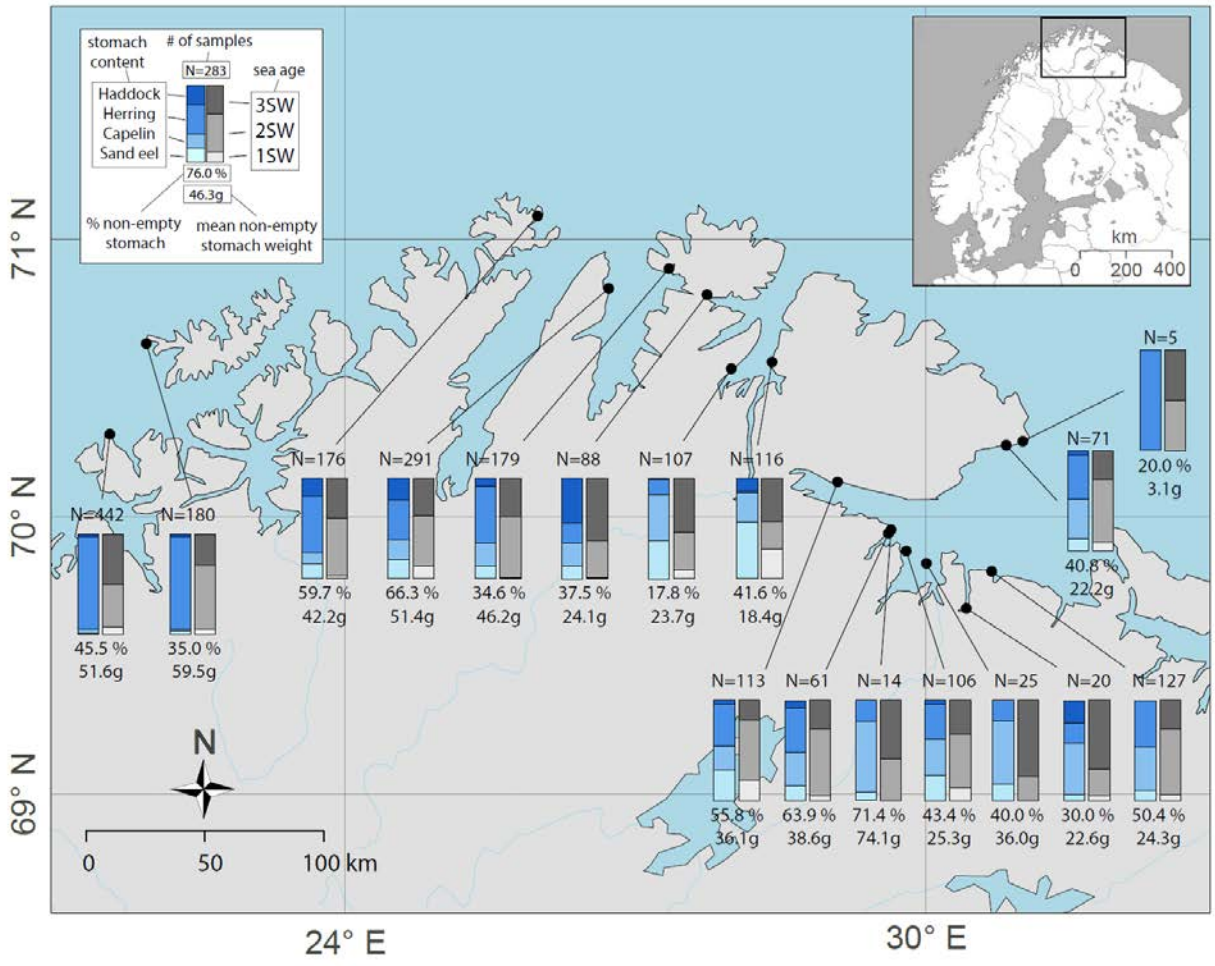

Figure 1: Map of the study region including sampling locations and a description of age and stomach content distributions and sample sizes in each sampling location. See the inset key for a description of the parameters of the spatial distributions given on the map.

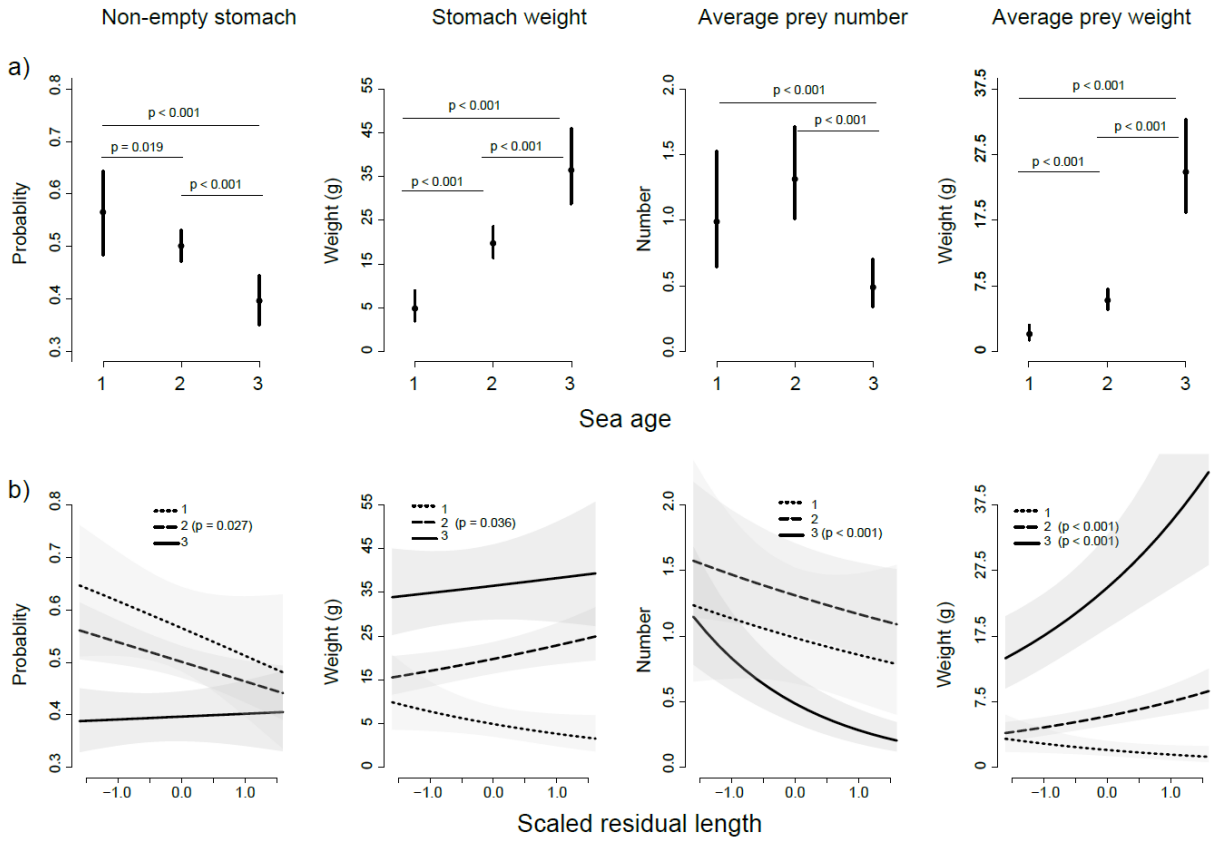

Figure 2: Changes in four stomach content variables in relation to sea age (a) and size at age (b) and associated 90% confidence intervals. Only p-values < 0.1 are given in the figures. Binned prey weights are given after back transformation to the original scale (i.e., grams). Note that estimates in the last three columns (stomach weight, average prey number and weight) are for non-empty stomachs only.

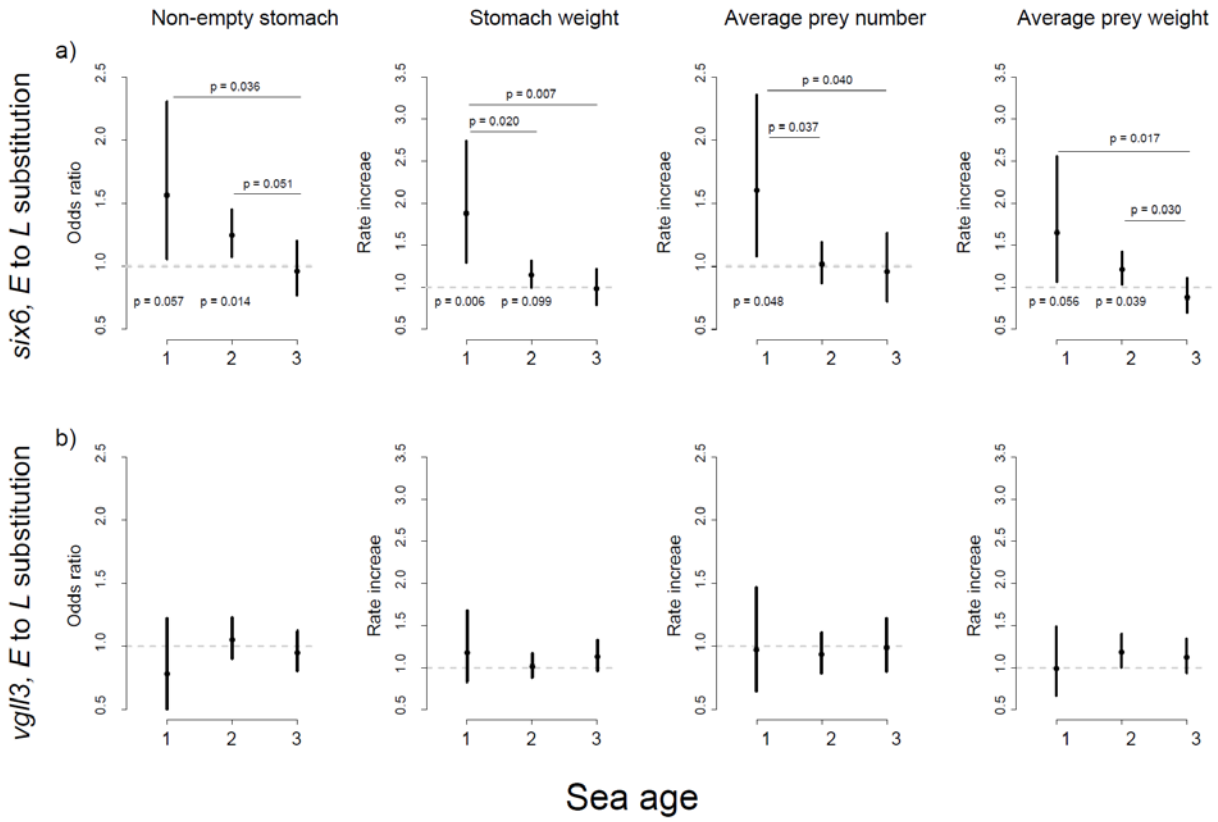

Figure 3: Changes in four stomach content variables for differing sea age classes in relation to allelic substation in *six6* (a) and *vgll3* (b) genomic regions and associated 90% confidence intervals. Only p-values < 0.1 are given in the figures. Note that estimates in the last three columns (stomach weight, average prey number and weight) are for non-empty stomachs only.

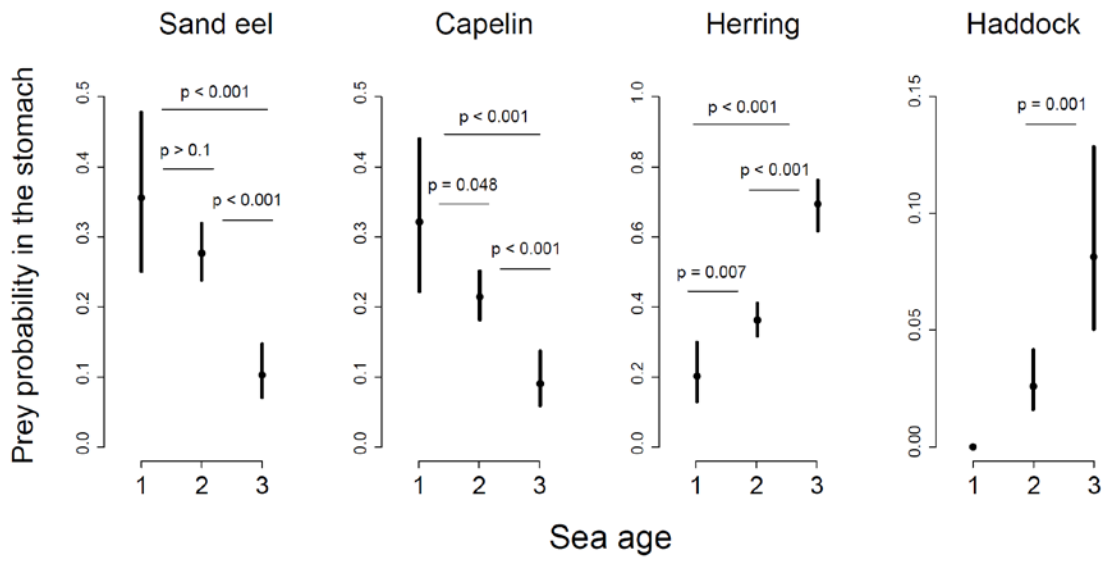

Figure 4: Relative prey composition for the four key prey species, measured as the proportion of each prey species contributing to the stomach content weight, in relation to age at maturity. Note that estimates include only non-empty stomachs.