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Rapid growth response of the Arctic charr to changing environmental conditions is not the result of a population bottleneck

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We tested if within-lake differences in individual growth in an Arctic charr population before and after nutrient enrichment was due to a population bottleneck, for example as a result of strong selection for growth in a limited part of the population. The positive change in growth pattern after treatment was not coincident with a reduction in microsatellite variability, and various genetic estimators of demographic change did not indicate changes in population size. This suggests that the change in growth was not a result of only a limited part of the population responding, but an overall response.

Introduction

Due to large-scale water regulation for hydroelectric purposes from the 1930s onwards, most lakes in the alpine region of northern Sweden have been under successive nutrient depletion — oligotrophication (Rydin *et al*. 2008). Among the negative consequences thereof are severely damaged fish populations. These negative consequences have also been observed in North America, while it has been shown in Canada (Ashley *et al*. 1997, Perrin *et al*. 2006) and in Sweden (Milbrink & Holmgren 1981, Rydin *et al*. 2008) that it is possible to mitigate these negative effects by compensatory nutrient enrichment, thus returning nutrient levels back to normal and thereby giving rise to much increased plankton development. In this way pelagic food-chains could be restored, providing sufficient food for pelagically feeding fish like Arctic charr (*Salvelinus alpinus*).

In a recent study nutrients were added to a regulated lake in northern Sweden, Stora Mjölkvattnet, hosting Arctic charr with clearly impaired growth, where fish were 70%–80% smaller by weight (about 30% smaller by length) in catchable age classes than fish from natural lakes in the same area (Milbrink *et al*. 2011). After only a few years of nutrient enrichment, the charr were roughly the same size for their age as before water regulation took place in 1942 (Rydin *et al*. 2008, Milbrink *et al*. 2011).

A response in growth rate at the population level might have two causes. Firstly, there is variation among individuals in the exploitation of the increased food availability. This could, for example, be a result of heterogeneity in food abundance, or variation in food processing efficiency among individuals. This variation could lead to rapid growth of some individuals, especially of those fish that later became can-

nibalistic. Thus, this could potentially result in a drastic reduction in population size, i.e. a bottleneck. Alternatively, all individuals respond to the increase in food availability in more or less the same way, which means that no sign of a bottleneck is observed. In this paper, we aimed at testing if a change in food availability due to fertilization resulted in a population bottleneck, or if it affected all individuals more or less the same way in a large population. We tested this by means of comparing genetic variation, as estimated by highly variable microsatellites, in two samples taken before and after the change in food availability.

Material and methods

General background

Lake Stora Mjölkvattnet is one of two deep, regulated lakes of medium-size in the upper reaches of the river Indalsälven in northern Sweden, in which the effects of large-scale application of nutrient enrichment have been studied. This lake was thus the target lake for nutrient application, while Lake Burvattnet located upstream was the untreated reference lake. Both lakes have an area of about 13.6 km^2, a maximum depth of 100–150 m and a regulation amplitude of 6–12 m (Rydin *et al*. 2008). The biological properties of the lakes are also similar. The experiment started in 2000 with two years of monitoring of chemical-physical and biological variables. From 2002 to 2009, lake Stora Mjölkvattnet was the object of yearly nutrient additions. General background information, objectives for and periodicity of fertilizer application, proportions between main constituents (P and N) and application procedures are presented in Rydin *et al*. (2008).

Fish populations in the lake system consist mainly of Arctic charr and brown trout (*Salmo trutta*) in about equal proportions, and some burbot (*Lota lota*). No other fish species are present. Fish composition is typical for lakes in this part of Scandinavia. Before nutrient addition, the fish were in poor condition and fish abundance was low due to the negative effects of water regulation. That situation still persists in the reference lake. Before regulation in 1942 the fish were undoubtedly in a good state (Milbrink *et al*. 2011). Nutrient enrichment started in June 2002. Fulton's coefficients (Ricker 1975), reflecting the condition of fish, largely increased from about 0.8 in August 2001 (poor condition) to ca. 0.95 in August 2003 (good condition), i.e. in little more than one year. Autumn test-fishing with bottom-set nets revealed that the sizes of most charr had greatly increased — all year-classes of charr increased in growth after nutrient enrichment, especially ages 5 to 8 (4+ to 7+) (Fig. 1).

Results from pelagic test-fishing in 2001 and 2005, showed the same general trend, i.e. increased Fulton's coefficients and increased growth rates of the charr (G. Milbrink unpubl. data). Echo-sound estimations and test-fishing data showed that there were on average more than twice as many fish in lake Stora Mjölkvattnet between 2006 and 2009 than before nutrient enrichment started or in the reference lake (G. Milbrink unpubl. data).

Sampling

Test-fishing was conducted on a regular basis in August (2000–2009) with standardized bottom-set multipanel survey gillnets of the Nordic type (Rydin *et al*. 2008, Milbrink *et al*. 2011), in accordance with EU-directives (CEN 2005). Complementary pelagic test-fishing was conducted in lake Stora Mjölkvattnet for two nights in August 2001 and in August 2005 (after 3 years of enrichment; Hammar & Filipsson 1985), respectively. These fishings took place above the maximum depth of the lake, using similar but deeper (6 m) multipanel survey nets of the Nordic type. The 6–12 m water stratum was fished during the first night and the 12–18 m water stratum during the second. Computerized echo-sounding surveys of fish abundance utilizing Simrad EY 500 echo-sound equipment were simultaneously performed. Measurements of length and weight, estimates of condition and state of maturity, and specific samples taken from the fish (for instance otoliths for age-determination) closely follow Milbrink and Holmgren (1981) and Milbrink *et al*. (2011). Fulton's formula for estimation of the condition (K) of the fish was used (Ricker 1975):

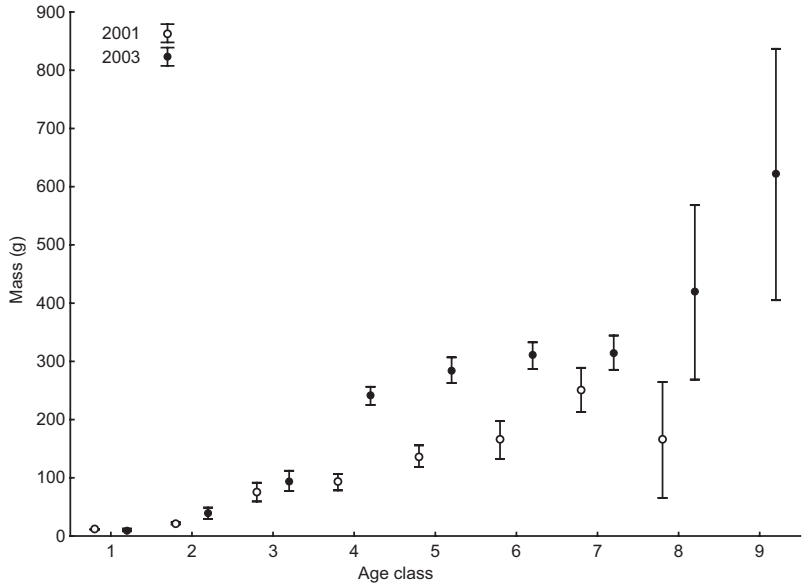

Fig. 1. Mean weights (± SE) of different age classes (+years) of Arctic charr caught in autumn test-fishings with bottom-set nets in lake Stora Mjölkvattnet before nutrient enrichment in 2001 (empty circles), and after enrichment in 2003 (filled circles). Growth curves differ significantly (factorial ANOVA on $\log_{10}$-transformed weights at age 1+ to 7+, whole model: $F_{13,135} = 77.5$, $r^2 = 0.87$, $p < 0.001$, sampling year effect: $F_{1,135} = 28.7$, $p < 0.001$, age x sampling year interaction: $F_{6,135} = 2.3$, $p = 0.042$).

$K = W \times 100/L^3$, where W is the weight (g) and L is the length (mm).

Samples for the DNA analyses were taken from Arctic charr caught in the pelagic test-fishing in 2005 (40 fish). Similar samples from charr caught in the autumn test-fishing with bottom-set nets were taken in 2001 (84 fish), as well as in 2005 (74 fish). Fish used for the DNA analyses were selected from four easily identifiable regions (based on morphometric characteristics) of the lake — about 15 fish from each region each year — also representing different depth-zones.

DNA was extracted from pieces of the fins by Chelex. We used the following primers: Sal61 (McGowan *et al*. 2004), Smm10, Smm22, and Smm24 (Crane *et al*. 2004), and Sto8 and MST85 (Primmer *et al*. 1999). The PCR conditions were as follows: Sal 61: 0.2 mM dNTP, 0.5 μM primer, 0.5 U AmpliTaq, with one cycle at 95 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, at 55 °C for 30 s, at 72 °C for 30 s, and finally at 72 °C for 7 min; Smm10, Smm22 and Smm24: 0.2 mM dNTP, 0.4 μM primer, 0.5 U AmpliTaq, with one cycle at 92 °C for 2 min, followed by 30 cycles at 92 °C for 15 s, at 55–56 °C for 15 s, at 72 °C for 30 s, and finally at 72 °C for 10 min; Sto8: 0.2 mM dNTP, 0.3 μM primer, 0.375 U AmpliTaq, with one cycle at 94 °C for 3 min, followed by 32 cycles at 94 °C for 1 min, at 62 °C for 1 min, at 72 °C for 1 min, and finally at 72 °C for 5 min; MST85 0.2 mM dNTP, 0.3 μM primer, 0.375 U AmpliTaq, with one cycle at 95 °C for 3 min, followed by 33 cycles at 94 °C for 1 min, at 58 °C for 1 min, 72 °C for 1 min, and finally at 72 °C for 5 min.

Genetic variability was estimated with FSTAT (Goudet 2001).

Based on comparisons of different methods, Spencer *et al*. (2000) concluded that the number of alleles (k) and gene diversity (or expected heterozygosity, H_e) are the most sensitive parameters with regard to bottlenecks. In a bottleneck, rare alleles are lost most quickly, which will reduce the number of alleles, and decrease heterozygosity. This can be summarized in the Garza-Williamsson test (Garza & Williamsson 2001), which utilises the fact that after a bottleneck alleles are lost in relation to their frequency, which is unrelated to the size range (R) of the marker. Thus, the probability that an allele is lost is independent of its size, and only dependent on its frequency. The Garza-Williamsson test statistic (GW) is then simply k/R (Garza & Williamsson 2001). In general, if there was a bottleneck due to strong selection, a consistent reduction in the number of alleles, in expected heterozygosity and of the GW index in all six loci will be seen.

Results and discussion

There was, on average, 18.7 alleles (SE = 4.14) in 2001, and 20.3 alleles (SE = 4.01) in 2005 among the six loci (Table 1). In five of the six loci, a slight increase in the allele number was found, but this was not statistically significant ($p = 0.75$, combined probability the χ^2-test for each locus). If allelic richness was used instead, which takes into account different sampling effort, the picture is more clear; three loci show an increase and three loci show a decrease in allelic richness ($p = 0.99$, combined probability of the χ^2-test for each locus). Gene diversity averaged over loci was 0.82 (SE = 0.046) in 2001, and 0.82 (SE = 0.040) in 2005 (Wilcoxon test: $p = 0.60$). The GW-statistic was 0.39 (SE = 0.06) in 2001 and 0.37 (SE = 0.08) in 2005 (Wilcoxon test: $p = 0.31$).

F_{IS} was 0.087 in 2001 ($p < 0.001$) and 0.106 in 2005 ($p < 0.001$), indicating consistent inbreeding in this population. A more plausible explanation is that since sampling was done from different parts of a relatively large lake, there might be unknown sub-structures of the Arctic charr population and the pooling of data thus creates a Wahlund effect.

The indices sensitive to bottlenecks, allelic diversity (or allelic richness), gene diversity and the GW index were similar and did not differ significantly or in any systematic way between years. This does not entirely rule out an effect of a bottleneck, which however could not have been large. Thus, a more plausible explanation is that the addition of nutrients acted more or less in the same way in the whole population, rather than being effective in a few individuals at the expense of others. Nutrient addition started in Lake Stora Mjölkvattnet in late June 2002 (Rydin *et al*. 2008). Already in the autumn test-fishing in mid-August of the same year — after less than two months — the charr in all age-classes showed accelerated growth, manifested in highly significant increases in both length and body mass (G. Milbrink unpubl. data). Comparisons may be made with the long-term fertilization project in the Kuparuk River, North Alaska, where Arctic grayling very rapidly responded positively to nutrient additions (Slavik *et al*. 2004; J. E. Hobbie pers. comm.).

Our results support the hypothesis that all individuals responded in more or less the same way rather than that there was a selection event. This interpretation is supported by the fact that the Arctic charr seems to have very low variability in growth trajectories (Björklund *et al*. 2003), which means that any change in food availability will affect all individuals in the same way. This conclusion is further supported by results from diet analyses showing that nearly all charr, irrespective of size and locality caught in test-fishing in the lake after the start of nutrient additions in 2002, had been feeding on the same food items in the zooplankton (G. Milbrink unpubl. data).

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Table 1. Basic population genetic statistics for the six loci analysed in 2001 and 2005. *n* is the sample size (*see* Material and methods for details).

Locus	Sample size (*n*)		Number of alleles		Allelic richness		Gene diversity	
	2001	2005	2001	2005	2001	2005	2001	2005
Smm10	81	110	8	12	7.0	9.1	0.72	0.71
Smm24	81	104	32	35	25.3	25.8	0.94	0.95
Sto8	54	101	16	18	14.1	13.7	0.75	0.70
Smm22	82	109	29	31	22.1	21.9	0.93	0.93
Sal61	72	93	10	12	8.8	8.7	0.73	0.75
MT85	48	37	17	14	15.0	14.0	0.86	0.87
Mean	70	92	18.7	20.3	15.38	15.53	0.82	0.82
SE			4.14	4.01	2.94	2.83	0.041	0.046

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