



## Review

Haemoglobin genotypes in cod (*Gadus morhua* L): Their geographic distribution and physiological significanceStine D. Ross<sup>a,\*</sup>, Jane W. Behrens<sup>a</sup>, Keith Brander<sup>a</sup>, Caroline Methling<sup>b</sup>, Jarle Mork<sup>c</sup><sup>a</sup> Technical University of Denmark, National Institute of Aquatic Resources, Charlottenlund Castle, 2920 Charlottenlund, Denmark<sup>b</sup> Marine Biological Section, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark<sup>c</sup> Department of Biology, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

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## ABSTRACT

Haemoglobin polymorphism in cod (*Gadus morhua* L) has been investigated throughout the last 50 years. Field studies have shed light on the geographic distribution of the two common alleles (*Hbl*<sup>1</sup> and *Hbl*<sup>2</sup>), and laboratory studies have shown effects of genotype on physiological traits such as growth, reproduction and hypoxia tolerance. The geographic distribution of alleles shows a correlation with temperature, with increasing frequency of *Hbl*<sup>1</sup> in warmer areas. This is likely due to temperature-related differences in oxygen affinity of the three genotypes. We provide a general ecological introduction to cod haemoglobin polymorphism and a detailed discussion of physiological studies, particularly laboratory growth studies. Although differences in oxygen uptake are almost certainly a contributory mechanism to observed differences in traits such as growth rate, many other environmental, behavioural and social factors may also contribute, making it difficult to quantify the effect of *Hbl* either experimentally or in the field.

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## Contents

1. Introduction . . . . .	158
2. Distribution . . . . .	160
3. Physiological performance . . . . .	161
3.1. Temperature preference . . . . .	161
3.2. Oxygen transport . . . . .	162
3.3. Hypoxia tolerance . . . . .	163
3.4. Growth . . . . .	163
4. Discussion and conclusion . . . . .	166
Acknowledgement . . . . .	167
References . . . . .	167

## 1. Introduction

Haemoglobin (Hb) polymorphisms are common in many fish species such as salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) (Buhler and Shanks, 1959). In the early 1960s, the Danish geneticist Knud Sick was the first to describe Hb polymorphism in whiting (*Merlangius merlangus*) and Atlantic cod (*Gadus morhua*) (Sick, 1961). In cod, two haemoglobin loci were distinguished using agar gel electrophoresis; *Hbl* and *HblII*.

Gene products of *Hbl*, predominating in adult cod, were polymorphic. Sick (1961) described a simple polymorphism at this locus with two alleles (*Hbl*<sup>1</sup> and *Hbl*<sup>2</sup>) segregating into three easily distinguished genotypes; the two homozygotes *Hbl*-1/1 and *Hbl*-2/2, and the heterozygote *Hbl*-1/2.

*Hbl*<sup>1</sup> and *Hbl*<sup>2</sup> are predominant throughout the species' range, but additional alleles exist (Frydenberg et al., 1965; Sick, 1965a,b; Husebø et al., 2004; Verde et al., 2006; Borza et al., 2009; Halldórsdóttir and Árnason, 2009a,b; Andersen, 2012). Furthermore, studies using high-resolution techniques like IF (Isoelectric Focusing) have reported a previously undetected banding variability that probably represents both different alleles at *Hbl* as well as other loci (Fyhn et

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**Table 1**

*Hbl*<sup>1</sup> frequencies for different geographical areas. Tabulated *Hbl*<sup>1</sup> values are averages for a given period and area. The data are aggregated in the most suitable way to reduce the number of samples, thus the exact same values may not be found in the original source. The latitude and longitude correspond to the approximate midpoint of each area. Bottom temperatures are averages for the period 1960–2010 taken from ICES.

Year	Month	<i>Hbl</i> <sup>1</sup> freq.	Latitude	Longitude	Bottom temperature	Source
<b>North America</b>						
1967–81	–	0.05	54	–57	0	Jamieson and Birley, 1989
1967–81	–	0.06	49	–56	0	Jamieson and Birley, 1989
1962	6	0.04	47	–52	0	Sick, 1965b
1967–81	–	0.1	47	–60	1.75	Jamieson and Birley, 1989
1967–81	–	0.07	42	–68	8	Jamieson and Birley, 1989
1963	1	0.07	39	–75	8	Sick, 1965b
1962	3	0.08	41	–70	8	Sick, 1965b
<b>Greenland</b>						
1969–74	3–7	0.09	74	–26	2	Jamieson and Birley, 1989
1961/63	9	0.01	66.5	–50.5	2	Sick, 1965b
<b>Barents Sea</b>						
1993	1	0.14	68	41	4	Fyhn et al., 1994
1993	1	0.08	70	40	4	Jørstad and Nævdal, 1994
1964–67	1/11	0.11	72	26.5	4	Møller, 1968
<b>White Sea</b>						
1974–80	6–8	0.16	65	35.5	2	Karpov et al., 1984
1974–80	9–11	0.16	65	35.5	2	Karpov et al., 1984
<b>Baltic Sea</b>						
1962	5	0.04	63	18	3	Sick, 1965a
1962	10	0.01	63	18	3	Sick, 1965a
1962	5	0.01	60	19	4	Sick, 1965a
1999	4	0.01	54.5	18.5	5	Pörtner et al., 2001
1999	4	0.1	54	14.5	5	Pörtner et al., 2001
1999	4	0.61	54	10.5	5	Pörtner et al., 2001
1968	4/7/8	0.27	54.5	14.5	5	Jamieson and Otterlind, 1971
1968	8	0.15	57	18.5	5	Jamieson and Otterlind, 1971
1968	12	0.43	55	14.5	5	Jamieson and Otterlind, 1971
1961–62	2–8	0.21	54.5	14	5	Sick, 1965a
1968	7–8	0.34	54.5	13.5	5	Jamieson and Birley, 1989
1962	3	0.03	55	19	5	Sick, 1965a
1961	11	0.04	56	18.5	5	Sick, 1965a
1961	10	0.03	59	21	5	Sick, 1965a
1968	10–12	0.51	57	18.5	5	Jamieson and Otterlind, 1971
1961–63	10–11	0.3	54.5	14	5	Sick, 1965a
1968	9–12	0.44	54.5	13.5	5	Jamieson and Birley, 1989
<b>Iceland</b>						
1968	12	0.4	66.5	–17	5.8	Jamieson and Jónsson, 1971
1969–74	2–12	0.19	66.5	–22.5	5.8	Jamieson and Birley, 1989
1969–74	2–12	0.11	66	–23	5.8	Jamieson and Birley, 1989
1969–74	2–12	0.32	66.5	–20	5.8	Jamieson and Birley, 1989
1969–74	2–12	0.3	66.5	–15.5	5.8	Jamieson and Birley, 1989
1969–74	2–12	0.61	63.5	–20	5.8	Jamieson and Birley, 1989
1969–74	2–12	0.09	63	–22	5.8	Jamieson and Birley, 1989
1969–74	2–12	0.1	64	–13	5.8	Jamieson and Birley, 1989

**Table 1 (continued)**

Year	Month	<i>Hbl</i> <sup>1</sup> freq.	Latitude	Longitude	Bottom temperature	Source
1968–69	3/4/6	0.16	64.5	–21.5	5.8	Jamieson and Jónsson, 1971
<b>Danish waters</b>						
1961	8	0.63	57	11	7	Sick, 1965a
1968	12	0.62	55	12	7	Jamieson and Otterlind, 1971
1961	2/4/8	0.61	55.5	11	7	Sick, 1965a
1993	6	0.59	58	8	7	Fyhn et al., 1994
1961	6–8	0.66	57	9	7	Sick, 1965a
1961–62	3/8	0.62	54.5	11	7	Sick, 1965a
1961	5	0.62	55.5	12	7	Jamieson and Birley, 1989
1961	11	0.58	55.5	11	7	Sick, 1965a
1961	11	0.62	55	12	7	Sick, 1965a
1994	10	0.59	55	10	7	Husebø et al., 2004
1994	10	0.67	55	12	7	Husebø et al., 2004
<b>Norwegian coast</b>						
1985–90	–	0.25	68.5	17.5	7.13	Dahle and Jørstad, 1993
2002	4	0.36	68	16	7.13	Dahle and Jørstad, 1993
1992	4	0.22	62	5	7.13	Fyhn et al., 1994
1994	4	0.39	63	5.5	7.13	Husebø et al., 2004
1965–67	2–4	0.17	67.5	14.5	7.13	Møller, 1968
1962	2–3	0.52	59	5	7.13	Frydenberg et al., 1965
1961–62	2–3	0.63	58	7	7.13	Frydenberg et al., 1965
1962	2–3	0.12	67.5	13.5	7.13	Frydenberg et al., 1965
1962	3	0.49	63.5	11.3	7.13	Frydenberg et al., 1965
1963	3	0.1	70.5	31.5	7.13	Frydenberg et al., 1965
1994	3	0.22	68.5	16.5	7.13	Husebø et al., 2004
1965–66	3	0.14	67	10.5	7.13	Møller, 1968
1965–66	3	0.23	69.5	17.5	7.13	Møller, 1968
1965–66	3	0.24	70.5	24.5	7.13	Møller, 1968
1987–90	3–4	0.21	67.5	14	7.13	Dahle and Jørstad, 1993
2002	3–4	0.23	71	27	7.13	Dahle and Jørstad, 1993
1992	3–4	0.12	67	12	7.13	Fyhn et al., 1994
1994	3–4	0.6	60	4	7.13	Husebø et al., 2004
1978/84	3–4	0.47	63.5	11	7.13	Mork and Sundnes, 1985
1966–67	3–4	0.27	62.5	6	7.13	Møller, 1968
1995–97	3–4	0.23	67.5	13.5	7.13	Nordeide and Båmstedt, 1998
2002–03	3–4	0.2	68	13.5	7.13	Wennevik et al., 2008
1992–93	4–5	0.57	60	6	7.13	Fyhn et al., 1994
1962	1	0.5	62	5	7.13	Frydenberg et al., 1965
1962	1	0.64	58.5	9	7.13	Frydenberg et al., 1965
2002	3–5	0.63	60	4	7.13	Dahle and Jørstad, 1993
1994	5	0.6	58.5	9	7.13	Husebø et al., 2004
1989–91	5–8	0.61	59	9.5	7.13	Gjøsæter et al., 1992
1977–83	10	0.5	63.5	11	7.13	Mork and Sundnes, 1985
1962	9	0.42	65	11	7.13	Frydenberg et al., 1965
1961–62	9	0.44	62.5	7	7.13	Frydenberg et al., 1965
1994	9	0.58	58.5	9	7.13	Husebø et al., 2004
1986–90	9–1	0.6	59	9.5	7.13	Gjøsæter et al., 1992
2001	9–11	0.43	67	13	7.13	Dahle et al., 2006
<b>Faeroe Waters</b>						
1961/63	4	0.07	61.5	–6	7.4	Sick, 1965b
1992	1	0.08	62	–7	7.4	Fyhn et al., 1994

(continued on next page)

Table 1 (continued)

Year	Month	Hbl <sup>1</sup> freq.	Latitude	Longitude	Bottom temperature	Source
1961–62	7–8	0.02	65.5	–21.5	7.4	Sick, 1965b
1971/ 1976	3–6	0.06	62	–7	7.4	Jamieson and Birley, 1989
1971/ 1976	3–6	0.19	60.5	–9	7.4	Jamieson and Birley, 1989
1961–62	5/8	0.02	64	–20	7.4	Sick, 1965b
1970/ 1976	–	0.6	0.35	–6	9.57	Jamieson and Birley, 1989
1971– 72/74/ 77	3	0.57	53.5	–5	10.56	Jamieson and Birley, 1989
1966	4/8	0.58	60	–1	7.62	Wilkins, 1969
North Sea						
1970	1	0.44	58	3	7.62	Jamieson and Thompson, 1972
1967–81	7	0.58	59.5	1.5	7.62	Jamieson and Birley, 1989
1970	6	0.63	58	–5	7.62	Jamieson and Thompson, 1972
1965	4	0.55	57	–2	8.56	Wilkins, 1967
1969	12	0.68	54	2	8.56	Jamieson and Thompson, 1972
1999	2	0.66	55.5	6.5	8.56	Pörtner et al., 2001
1966	2	0.54	57	–2	8.56	Wilkins, 1967
1966–67	2/4/6	0.6	56	–4.5	8.56	Wilkins, 1969
1967–81	2–3	0.67	55	2	8.56	Jamieson and Birley, 1989
1961–63	2–9	0.62	55.5	6.5	8.56	Sick, 1965b
1967–81	1/11	0.67	59.5	1.5	8.56	Jamieson and Birley, 1989
1962	7	0.65	57	2	8.56	Sick, 1965b
1966	9	0.6	57	–2	8.56	Wilkins, 1967
1969–70	12–1	0.53	52	–2.5	11.62	Jamieson and Thompson, 1972
1968/70	2	0.66	53	3	11.62	Jamieson and Thompson, 1972
1967–81	2–4	0.63	52	2.5	11.62	Jamieson and Birley, 1989
1963	1	0.72	52	3	11.62	Sick, 1965b
1967–81	10–12	0.6	52	2.5	11.62	Jamieson and Birley, 1989
1968	12	0.73	50.5	–2.5	12.23	Jamieson and Thompson, 1972
1966	2	0.5	50	–4	12.23	Wilkins, 1969
1971	3	0.6	50.5	0	12.23	Jamieson and Birley, 1989

al., 1994; Husebø et al., 2004). Among the reported rare genotypes, some appear to be more abundant in the far northern parts and others in the southern parts of the cod's range (Frydenberg et al., 1965; Sick, 1965b; Møller, 1968; Husebø et al., 2004; Andersen, 2012). Most recent papers do not show photos of gels with the actual *Hbl* genotypes as they appear on different electrophoretic platforms. Therefore the respective interpretations of the *Hbl* banding patterns cannot be cross-checked.

The genetic and molecular basis of the *Hbl* protein variants and the link between the genotype and the physiological functions of the *Hb* gene products have been described in detail by Andersen et al. (2009) and Andersen (2012). For a detailed biochemical and molecular background to cod haemoglobin, we refer to the review by Andersen (2012).

The observed *Hbl* genotypic distributions with *Hbl*<sup>1</sup> decreasing from south to north (Frydenberg et al., 1965; Sick, 1965a,b; Karpov and Novikov, 1980; Mork and Sundnes, 1984; Husebø et al., 2004; Andersen et al., 2009) have been associated with both geographic, between stock, differences in environmental temperatures and with individual, within stock, temperature preferences on a more local scale (Karpov and Novikov, 1980; Petersen and Steffensen, 2003; Behrens

et al., 2012). Both explanations have been anchored to genotypic differences in physiological performance (e.g., Mork et al., 1983; Brix et al., 1998; Pörtner et al., 2001) and strongly suggest that cod haemoglobins are affected by natural selection (Kirpichnikov, 1981; Mork and Sundnes, 1985; McFarland, 1998; Andersen et al., 2009; Halldórsdóttir and Árnason, 2009a). Natural selection and behavioural habitat preferences are complementary explanations; environmental barriers may have led to genetic differentiation through local adaptations, and behaviour could potentially help maintain the variation. An example of this was recently demonstrated for the Icelandic cod stocks, where genetic differentiation (Pan I and microsatellite) was correlated with homing preferences for distinct spawning areas, i.e., preference for the warmer south-western areas or the cooler north-eastern areas (Pampoulie et al., 2011).

Physiological performance and aerobic capacity are determined by the ability to bind and unload oxygen. Haemoglobins with a high affinity bind O<sub>2</sub> at lower ambient levels, and the partial pressure at which haemoglobins are half saturated, P<sub>50</sub>, is low. However, high O<sub>2</sub> affinity entails a matching reduction in the partial pressure of oxygen in the tissue for unloading of O<sub>2</sub> at the tissues.

Laboratory studies investigating the relationship between *Hbl* polymorphisms, temperature preference and physiological performance are inconsistent. Differences in experimental design and methodology between studies may influence the outcome of the experiments, leading to results which in some cases appear to contradict the expectations arising from studies of geographical allele frequency distributions. Possible reasons for such inconsistencies need to be explored in order to move towards a more comprehensive understanding of the significance of the *Hbl* polymorphisms. Epigenetic and compensatory mechanisms influence physiological performance, thereby making it difficult to distinguish the effect of haemoglobin from other factors.

This review presents and discusses laboratory studies of the three main cod *Hbl* genotypes, examining the experimental design, methods and conclusions drawn. The observed allelic distribution in natural populations is analysed in relation to temperature, and the physiological performance of the different haemoglobin genotypes is discussed. Proposals for future research are presented and evaluated.

## 2. Distribution

*Hbl* allele frequencies, which were related to latitude in the early studies (Frydenberg et al., 1965; Sick, 1965a,b), have been corroborated in more recent reports (e.g., Karpov and Novikov, 1980; Mork and Sundnes, 1984; Husebø et al., 2004; Andersen et al., 2009). However it is evident that latitude *per se* has no effect on *Hbl* allele frequency and that the observed clines are due to water temperature (Pörtner et al., 2001).

Cod homozygous for *Hbl*<sup>2</sup> are termed the *cold-water* type and cod homozygous for *Hbl*<sup>1</sup> the *warm-water* type (Frydenberg et al., 1965; Pörtner et al., 2001). With the possible exception of Baltic Sea cod, which is also the most genetically divergent of all cod stocks (Mork et al., 1985), the temperature related cline in cod *Hbl* allele frequencies appears consistent in studies published so far, and applies to both sides of the North Atlantic (Table 1).

In some areas, e.g., along the Norwegian coast (Møller, 1966, 1968; Dahle and Jørstad, 1993; Nordeide and Båmstedt, 1998; Wennevik et al., 2008) and in Icelandic waters (Jamieson and Jónsson, 1971; Jamieson and Birley, 1989), intermingling of populations with different *Hbl*<sup>1</sup> frequencies occurs during the spawning season, thus changing the prevailing *Hbl* pattern. The North East Arctic cod stock migrates from the cold Barents Sea to spawn in areas along the Norwegian coast in February to April and this gives rise to a marked decrease in the *Hbl*<sup>1</sup> frequency in those locations (Fig. 1). The noteworthy heterogeneity seen in the Icelandic waters (Table 1) may be explained by the existence of two genetically differentiated spawning components with one component preferring the warmer waters southwest of Iceland and the other

the cooler waters in the northeast (Pampoulie et al., 2011). In other areas, where seasonal cod migrations are small and do not involve large temperature differences, e.g., the North Sea, there is no obvious seasonal change in local *Hbl<sup>I</sup>* frequency (Fig. 2).

Overall, the *Hbl* allele frequencies for the entire range of cod show an increase in *Hbl<sup>I</sup>* with increasing temperature (Figs. 3, 4). However, the relationship between temperature and *Hbl<sup>I</sup>* appears to be stepped, with the proportion of *Hbl<sup>I</sup>* below 0.2 up to 4 °C, above 0.5 at temperatures above 8 °C and highly variable in the temperature range 5–8 °C. While this may in part be explained by the kind of seasonal differences in *Hbl<sup>I</sup>* frequency observed at the Norwegian coast due to migration, it may also indicate that other genetic and environmental factors can influence the distribution of the *Hbl* genotypes.

In some studies on the population structure of cod, authors have interpreted the geographic *Hbl* allele frequency differences as signs of a complex genetic subdivision into separate and isolated populations (Dahle and Jørstad, 1993). However, if temperature is acting selectively then the utility of this locus as a neutral population marker is questionable (Mork et al., 1984b; Mork and Sundnes, 1985).

In the early juvenile stages of cod gene products of *HbII* predominate and *Hbl* genes do not become active until the fish are 3–4 cm Fyhn et al. (1995). The authors hypothesised that the change in gene expression could be related to adaptation to a new environment, i.e., the shift from pelagic to demersal life style.

### 3. Physiological performance

Temperature is regarded as ‘the ecological master factor’ of ectothermal organisms (Brett, 1971) as it influences all aspects of their physiology (Lefrancois and Claireaux, 2003; Pörtner and Knust, 2007). Growth rate is maximised within a species-specific range of temperatures, often referred to as the optimal temperature for growth ( $T_{opt}$ ), which for satiation fed, maturing and adult Atlantic cod kept at normoxic conditions ranges between 11 and 14 °C (Jobling, 1983; Claireaux et al., 2000; Colosimo et al., 2003; Brix et al., 2004; Jordan et al., 2006). However  $T_{opt}$  declines with increasing size in cod and is, thus, much higher in smaller fish (Pedersen and Jobling, 1989; Björnsson and Steinarsson, 2002).  $T_{opt}$  is also reduced when food is limited because maintenance costs are higher at high temperatures (Brett, 1971). It is noteworthy that growth rates and condition factors increase with temperature over the actual thermal range occupied by cod in the wild and are highest in the warmest areas in which they occur (Dutil and Brander, 2003; Rätz and Lloret, 2003).

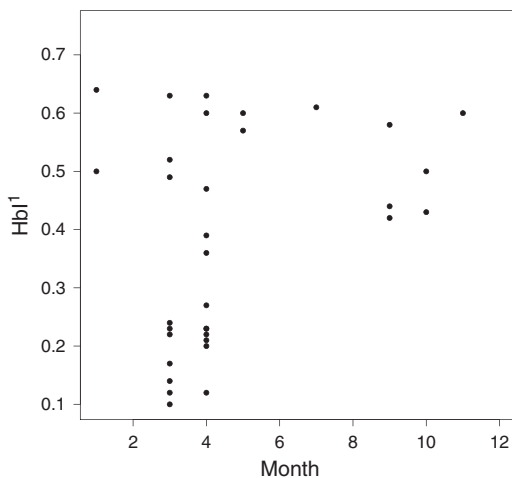


Fig. 1. Monthly *Hbl<sup>I</sup>* allele frequencies along the Norwegian coast. The *Hbl<sup>I</sup>* frequencies are based on reported cod blood sampling from a wide range of authors (see Table 1 for a complete table with *Hbl<sup>I</sup>* frequencies).

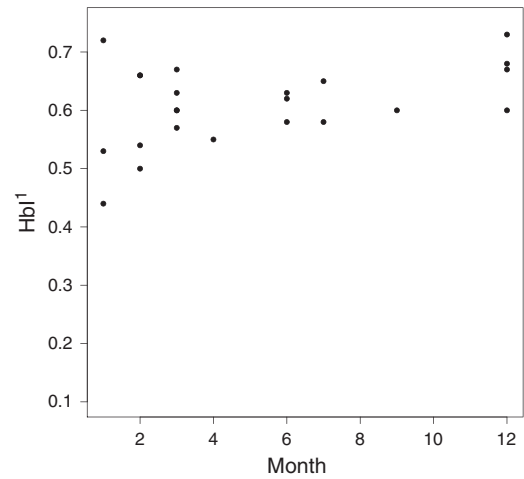


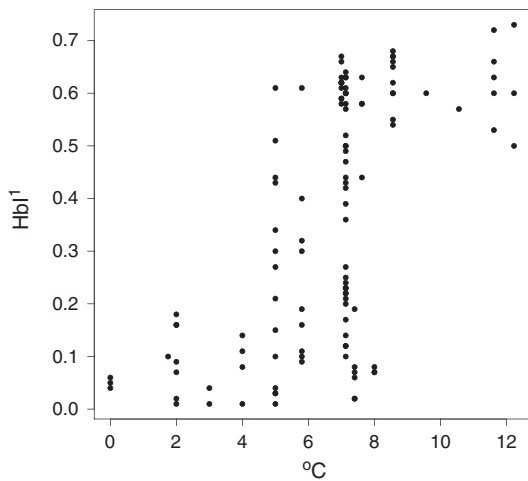
Fig. 2. Monthly *Hbl<sup>I</sup>* allele frequencies in the North Sea. The *Hbl<sup>I</sup>* frequencies are based on reports by a wide range of authors, while the annual average bottom temperatures are adopted from other sources as well (see Table 1 for a complete table with *Hbl<sup>I</sup>* frequencies and the matching bottom temperatures).

The reported  $T_{opt}$  found in laboratory experiments with satiation fed cod is higher than observations from the field; a comprehensive tagging study encompassing thermal history data (>90 days) for more than 300 cod from 8 geographical areas demonstrated that maximum growth rates were obtained by fish having a mean thermal history of 8–10 °C (Righton et al., 2010). This is probably due to differences in feed intake, but it is a mistake to regard it as evidence of food limitation, since the satiation fed experimental environment (or the environment of a fish farm) is an abnormal one.

#### 3.1. Temperature preference

In theory, the preferred temperature of a fish ( $T_{pref}$ ) should reflect the optimal temperature for growth ( $T_{opt}$ ), but thermoregulatory behaviour may not always ensure this (Jordan et al., 2006; Behrens et al., 2012). Basically, temperature preference strategies are extremely complex and vary with the physical environment (e.g., oxygen availability, Schurmann and Steffensen, 1992; Petersen and Steffensen, 2003) and internal factors/physiological state of the fish (e.g., digestive status (Gräns et al., 2010), size and age (Pedersen and Jobling, 1989; Swain and Kramer, 1995; Glover et al., 1997; Björnsson and Steinarsson, 2002; Gräns et al., 2010; Behrens et al., 2012)). Selecting a colder environment increases the blood oxygen affinity that increases the oxygen binding at the gills but may lower the oxygen unloading at the tissues. It also lowers the metabolism. These conditions favour energy conservation but not digestion, growth and fast swimming (Brett, 1971). Thus, there may be a trade-off between activities like feeding, digestion, predator avoidance and migration when selecting a thermal environment.

The geographic distribution of the two major haemoglobin alleles indicates that *Hbl-1/1* is adapted to warm waters and *Hbl-2/2* to cold waters (Sick, 1965a; Pörtner et al., 2001; Brix et al., 2004). Two separate studies have investigated the preferred temperatures of the *Hbl* types, with partially discrepant results. Petersen and Steffensen (2003) observed a significant and rather pronounced difference between the two homozygotes with *Hbl-1/1* showing preference for temperatures of  $15.4 \pm 1.1$  °C and *Hbl-2/2* for temperatures of  $8.2 \pm 1.5$  °C when kept under normoxic conditions. During hypoxia (35% air saturation),  $T_{pref}$  for *Hbl-1/1* fish fell to  $9.8 \pm 1.8$  °C, whereas no change was observed in *Hbl-2/2* fish (Petersen and Steffensen, 2003). The authors, however, hypothesised that a similar decrease in  $T_{pref}$  of *Hbl-2/2* might be observed if the oxygen levels were further reduced (<33%).



**Fig. 3.** *HbI<sup>1</sup>* frequency as a function of bottom temperature. The *HbI<sup>1</sup>* frequencies are based on reports by a wide range of authors, while the annual average bottom temperatures are adopted from other sources as well (see Table 1 for a complete table with *HbI<sup>1</sup>* frequencies and the matching bottom temperatures).

The observed dissimilarities in  $T_{\text{pref}}$  were recently confirmed by Behrens et al. (2012); *HbI-2/2* preferred temperatures of  $8.9 \pm 0.2$  °C, consistent with the findings by Petersen and Steffensen (2003), but *HbI-1/1* preferred substantially lower temperatures ( $11.0 \pm 0.6$  °C) than reported by Petersen and Steffensen (2003), yet still significantly higher than the value for *HbI-2/2* cod within the same study. The discrepancy between the two studies might be due to different methodologies, shuttle box versus an annular preference chamber. Furthermore, based on PCR and SNPs (single nucleotide polymorphisms), Behrens et al. (2012) observed that 17% of the experimental fish had recombinant haplotypes that were not compatible with any of the *HbI* genotypes, and it is possible that these haplotypes disturbed the overall pattern of temperature preference in both studies. Similar biases have been emphasised by other studies (Brix et al., 1998; Husebø et al., 2004; Andersen et al., 2009; Borza et al., 2009). Heterozygotes display less distinct temperature preferences, not significantly different from any of the homozygotes, and with greater inter-individual variation (Behrens et al., 2012). Furthermore, heterozygotes have a high degree

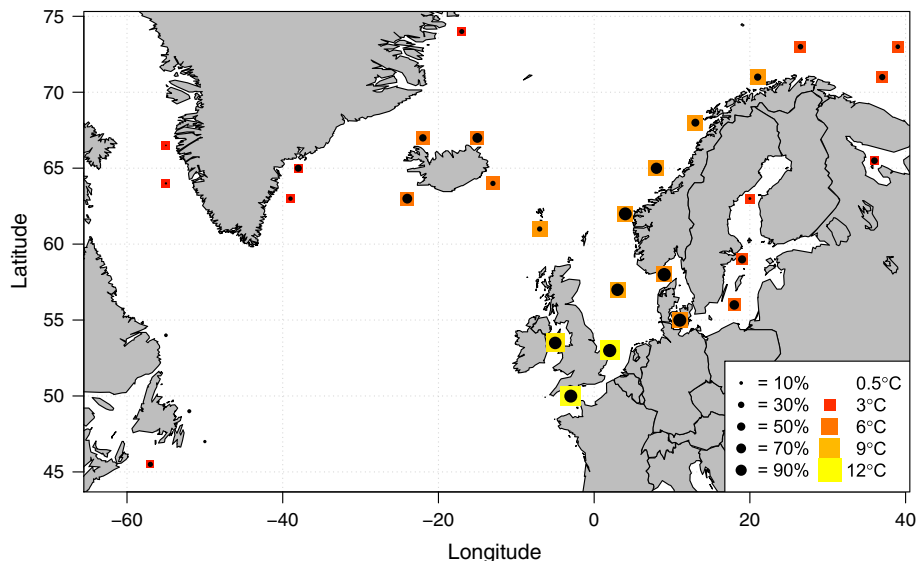
of plasticity in their expression of the two haemoglobins (Brix et al., 2004; Andersen et al., 2009; Borza et al., 2009) and are, thus, likely to thrive in a broader thermal range.

### 3.2. Oxygen transport

The earliest study on the *in vitro* oxygen affinity of the three common cod *HbI* genotypes was performed on intact erythrocytes and showed highest oxygen affinities for *HbI-2/2* and *HbI-1/1* at temperatures below and above 15 °C, respectively (Karpov and Novikov, 1980). These genotype-specific oxygen affinities have later been confirmed by other *in vitro* studies performed on haemolysates, although with less pronounced differences (Brix et al., 1998; Pörtner et al., 2001). However, in an *in vitro* study of haemolysates by McFarland (1998), a slightly different result was obtained: in fish acclimated to 10 and 16 °C, no genotypic differences were found, but at an acclimation of 4 °C, *HbI-2/2* had a higher  $O_2$  affinity. Similarly, when 10 °C acclimated fish were exposed to 4 and 16 °C, the *HbI-2/2* had higher  $O_2$  affinity. If the fish were allowed no acclimation time prior to experiments, a steady decrease in the affinity of *HbI-2/2* between 4 to 16 °C was evident. The  $O_2$  affinity of *HbI-1/1* was shown to be less temperature sensitive, and in later molecular studies this type is referred to as the temperature-insensitive haplotype (Andersen et al., 2009; Andersen, 2012).

Haematocrit, the ratio between erythrocytes and blood plasma, is also relevant in relation to physiological performance as more erythrocytes will enhance the blood oxygen carrying capacity. Mork and Sundnes (1984) investigated haematocrit in juvenile and adult cod from a Norwegian fjord and found higher haematocrit values in males homozygous for *HbI<sup>2</sup>*, but no differences between genotypes for females. Their results were later supported by McFarland (1998) who found higher haematocrit values in adult *HbI-2/2* at 5 and 10 °C, regardless of sex. In addition, when fish were allowed a period of 4 weeks acclimation prior to the blood sampling, the *HbI-2/2* type was also observed to have a higher haemoglobin concentration compared to the *HbI-1/1* type (McFarland, 1998).

Despite small dissimilarities in the above-mentioned studies, it appears that *HbI-2/2* has superior oxygen transport capabilities at low ambient temperature especially under hypoxic conditions, which is also reflected in the distribution of the *HbI<sup>2</sup>* allele.



**Fig. 4.** *HbI<sup>1</sup>* frequencies of cod in the North Atlantic. *HbI<sup>1</sup>* frequencies are shown with different-sized circles and bottom temperatures are shown as squares with a colour gradient. Both variables are based on the values shown in Table 1. For simplicity, some of the values have been aggregated according to area.

### 3.3. Hypoxia tolerance

Having a high oxygen affinity is considered advantageous for individuals encountering hypoxic conditions (Brix et al., 1998). The critical oxygen saturation ( $S_{crit}$ ) can be defined as the  $O_2$  level where a fish can no longer maintain an oxygen uptake that supports the standard metabolic rate (SMR; the oxygen uptake of resting, fasting fish) and can as such be used as a measure of hypoxia tolerance. Few studies have investigated the response of the *Hbl* genotypes to hypoxia, and with ambiguous results. The first study was conducted on wild-caught adult cod that were allowed at least 4 weeks of acclimation to 4, 10 or 16 °C (McFarland, 1998). No differences were observed in  $S_{crit}$  values at 10 and 16 °C, but at 4 °C, *Hbl*-2/2 had a lower  $S_{crit}$ . This also coincided with a significantly lower SMR in *Hbl*-2/2 at this temperature. When fish acclimated to 10 °C were exposed to 4 and 16 °C, the lowest  $S_{crit}$  was seen in *Hbl*-2/2 and *Hbl*-1/1, respectively. McFarland (1998) concluded that *Hbl*-2/2 is more tolerant to hypoxia but only when the fish are in a resting, fasting state that is supporting their SMR.

Two separate studies on juvenile offspring from wild-caught cod acclimated for either 1 or 5 weeks, failed to find any differences between the two *Hbl* types in the avoidance of hypoxic environments, ventilation rates or cortisol levels (Skjæråsen et al., 2008; Gamperl et al., 2009). However, Gamperl et al. (2009) observed *Hbl*-1/1 to have a lower  $S_{crit}$  than the other homozygote, suggesting that this genotype as opposed to the above-mentioned findings is more tolerant of hypoxia (Gamperl et al., 2009). Conversely, Methling et al. (2010) observed higher plasma cortisol and lactate levels in *Hbl*-1/1, after hypoxia exposure indicative of an increased level of stress in wild-caught juveniles allowed 3 weeks of acclimation. Petersen and Steffensen (2003) also hypothesised that *Hbl*-2/2 is more tolerant to hypoxia based on their observation that this genotype did not shift its  $T_{pref}$  at hypoxia. Altogether the above suggests that stress response and the associated metabolic adjustments may be *Hbl*-dependent – although it is not yet clear how and to what degree.

The apparent lack of a clear correlation between oxygen affinity and hypoxia tolerance may be partly due to the fact that *in vitro* studies of  $O_2$  affinity do not take into account the possibility of *in vivo* compensatory mechanisms. These can be of behavioural character e.g., avoidance of hypoxic habitats, increased ventilation, reduced activity levels or cessation of feeding (McFarland, 1998; Gamperl et al., 2009), and/or related to cellular compensatory control of Hb- $O_2$  affinity. Here organic phosphates (in fish mainly ATP and GTP) can be of major adaptive significance as these, when bound to the Hb, lower the  $O_2$  affinity (Weber and Jensen, 1988; Jensen et al., 1998). Thus, reducing the level of red blood cell organic phosphates is a mean to increase blood oxygen affinity during hypoxia (Val, 2000; Nikinmaa, 2001), which has also been documented in cod by McFarland (1998), who found a decrease in  $P_{50}$  concurrently with a decrease in ATP and GTP levels, thereby increasing the oxygen uptake in the blood. In conclusion, compensatory mechanisms are likely to reduce the genotypic differences, indirectly suggesting that the genotypes are in fact exceptionally flexible.

### 3.4. Growth

The growth rate and growth potential of a fish are determined by its developmental stage, genotype and environment (e.g., food availability and quality, temperature and oxygen availability). Separating the effects of each of these factors is ultimately impossible, because they interact antagonistically or synergistically, and this is one highly plausible reason for the inconsistent and contradictory results obtained in studies dealing with growth of cod with different haemoglobin genotypes. Conclusions drawn from comparing length and weight data from the field are also restricted by limited knowledge of the environmental and physiological history of the fish. Field estimates of growth show general trends for individuals or populations living in a particular area and under a more or less well-known set of

environmental conditions. Differences in growth rate between cod stocks were documented as early as the 1930s, when the Norwegian coastal cod were observed to grow faster and mature earlier than the Northeast Arctic cod (Rollefsen, 1934; Hysten, 1964). However, a common-garden experiment by Godø and Moksness (1987) demonstrated that fish from these two stocks grow and mature at the same rates under identical environmental conditions and this is confirmed by other studies (e.g., Taranger et al., 2006).

Some field studies, comparing lengths of cod with different genotypes, have shown growth-related differences whereas others have not (Sick, 1965a; Gjørseter et al., 1992; Jørstad and Nævdal, 1994). Higher growth rates of *Hbl*-2/2 have been reported in colder areas with temperatures closer to the genotype's  $T_{pref}$  (Mork et al., 1983; Mork and Sundnes, 1984; Mork et al., 1984a,b). For instance, in the Baltic Sea where the temperature and *Hbl*<sup>I</sup> frequency decrease from west to east, a similar decrease in length and weight of *Hbl*-1/1 is seen (Pörtner et al., 2001). In the southern part of the North Sea, where *Hbl*<sup>I</sup> is the predominant allele, *Hbl*-1/1 fish are generally larger than *Hbl*-2/2 fish (Pörtner et al., 2001). In conclusion, *Hbl*-2/2 appears to be superior to *Hbl*-1/1 at colder temperatures, and vice-versa (Mork and Sundnes, 1984).

Comparing results and drawing conclusions from laboratory studies are made difficult by the diversity of methodology, duration, acclimatisation period, tank environment, temperature regime, photoperiod, feed, and so forth. We assume that *Hbl* genotypes, according to Sick (1961), were correctly identified by the various electrophoretic techniques employed in the different studies, and, thus, that the observed inconsistencies are caused by other issues. An overview of these as well as other physiology studies is given in Table 2.

The most commonly drawn conclusion from laboratory studies is that no *Hbl* genotypic differences in growth exist (Colosimo et al., 2003; Gjerde et al., 2004; Jordan et al., 2006; Jørstad et al., 2006). The varieties of experimental design may, nevertheless, mask the actual effects of *Hbl*. Two studies show significant differences; Nævdal et al. (1992) found higher growth rates for *Hbl*-2/2 at all temperatures (6–14 °C), whereas Imsland et al. (2004) observed highest growth rates in *Hbl*-2/2 at high temperatures (13–16 °C) and in *Hbl*-1/1 at low temperatures (7 °C). The results obtained in the latter study deviates from all earlier findings regarding allelic distribution,  $T_{pref}$  and  $O_2$  affinity. The difference in growth could possibly be caused by a sibling or pedigree effect as the experimental animals were offspring from wild-caught fish and if a skewed proportion of the juveniles originate from the same parents, they are likely to be more similar in physiological performance. Glover et al. (1997) observed a tendency for higher growth in *Hbl*-2/2 at 12 °C and in *Hbl*-1/1 at higher temperatures, but the results were not significant. A fourth study, showed the opposite pattern: *Hbl*-1/1 grew faster than *Hbl*-2/2 when fed in excess (Gamperl et al., 2009). Reducing the amount of food removed this discrepancy (Gamperl et al., 2009).

Most of the laboratory studies selected juveniles randomly without considering the family relations. In a more recent study conducted by Gamperl et al. (2009) two heterozygotes were crossed and the offspring used for the experiments – thereby reducing the amount of unknown genetic factors potentially influencing the experiments. In this study *Hbl*-1/1 was observed to grow faster throughout the first 9 months when kept at 10 °C and fed in excess. Gjerde et al. (2004) also used full-sib families in their study, and although they did not find differences in growth, they observed a relatively high degree of heritability of growth (body weight), i.e., siblings resembled each other. They hypothesised that additive genetic effects (other than haemoglobin) likely influenced the results. On the contrary, Jørstad et al. (2006) were unable to distinguish between effects of genotype and family in their experiments. To disentangle this, future experiments should be based on well-defined families preferably with heterozygotic parents.

**Table 2**  
Overview of experimental and field studies on cod *Hbl*.

Study	Focus	Stage	Area	N	Results	Authors' Comments	Reference
Lab	O <sub>2</sub> -affinity 0–20 °C <i>In-vitro</i>	Adults	White Sea	–	<i>Hbl-2/2</i> have higher O <sub>2</sub> -affinity <15 °C and <i>Hbl-1/1</i> > 15 °C		Karpov and Novikov (1980)
Field	Maturation	Adults	Trondheims fjord	276	<i>Hbl-2/2</i> males mature earlier <i>Hbl-2/2</i> females have more developed gonads early in the season	Results are likely secondary effects of growth	Mork et al. (1983)
Field	Haematocrit	Juv., adults	Trondheimsfjord	149	<i>Hbl-2/2</i> males have higher haematocrit <i>Hbl-2/2</i> juvenile males are larger	Large seasonal temperature fluctuations may influence the result	Mork and Sundnes (1984)
Field	Growth Maturation	Adults	Trondheimsfjord and Olso fjord	–	<i>Hbl-2/2</i> are larger than <i>Hbl-1/1</i> (males: <i>Hbl-2/2</i> > 1/2 > 1/1, females: <i>Hbl-1/2</i> > 2/2 > 1/1) Male <i>Hbl-2/2</i> mature earlier	Size-selective fishing will tend to level out potential genotypic differences	Mork et al. (1984b)
Field	Growth Genotypic frequency	Im-mature	Trondheimsfjord	119	<i>Hbl-2/2</i> are largest, <i>Hbl-1/1</i> smallest Year-class variation	<i>Hbl</i> unreliable as a population marker	Mork et al. (1984b)
Lab	Growth 6, 10, 14 °C	Larvae	Farmed (origin: Bergen)	100 per exp.	<i>Hbl-2/2</i> have highest growth rate at all temperatures <i>Hbl-1/1</i> have lowest	Perhaps due to differences in metabolic capacity	Nævdal et al. (1992)
Field	Growth	Juv., adults	Skagerrak	12–1300	No differences	The benefit of being of a particular genotype may be temperature dependent	Gjøsæter et al. (1992)
Field	Growth	Juv. 4–24 months	Tromsø/ Bergen and Barents Sea	Ca. 2500	Only significant differences ( <i>Hbl-2/2</i> larger) in 1 out of 10 samples (consisting of 281 fish)		Jørstad and Nævdal (1994)
Lab	Growth 12, 14, 16, 18, 20 °C 3 diff. light regimes	Juv.	Bergen	50 per exp.	A non-significant tendency for higher growth in <i>Hbl-2/2</i> at 12 °C and for <i>Hbl-1/1</i> at higher temperatures	Perhaps significant differences if the O <sub>2</sub> sat. level was less favourable	Glover et al. (1997)
Lab	O <sub>2</sub> -affinity 10, 15 and 20 °C <i>In-vitro</i>	Adults	Bergen	60–70 per exp.	<i>Hbl-2/2</i> have higher O <sub>2</sub> -affinity at 10–15 °C and <i>Hbl-1/1</i> at 20 °C	Perhaps due to differences in metabolic capacity	Brix et al. (1998)
Lab	O <sub>2</sub> -affinity 5, 10, 16 °C <i>In-vitro</i> Hypoxia tolerance Haematocrit	Adults	Øresund, Denmark and Lowestoft, Norway (Haemato-crit)	40–70	<i>Hbl-2/2</i> have higher O <sub>2</sub> -affinity at 4 °C (acclimation) and at 4 and 16 °C (acute exposure) <i>Hbl-2/2</i> have lower S <sub>crit</sub> at 4 °C and <i>Hbl-1/1</i> at 16 °C <i>Hbl-2/2</i> have higher haematocrit at 5 and 10 °C	<i>Hbl-2/2</i> has superior oxygen transport at low temperatures which supports the observed increase in <i>Hbl</i> <sup>2</sup> with latitude	McFarland (1998)
Lab	Competition Feeding behaviour	1-group	Farmed 6–7 °C (origin: W. Norway)	29	<i>Hbl-2/2</i> have highest capture success and take prey first	When fish have to compete for food <i>Hbl-2/2</i> have an advantage	Salvanes and Hart (2000)
Field/ Lab	Growth (field) O <sub>2</sub> -affinity (lab) 4, 8 °C <i>In-vitro</i>	Adults	North Sea and Baltic Sea	803: field 30: lab	<i>Hbl-2/2</i> are largest in the Baltic Sea and smallest in the North Sea <i>Hbl-2/2</i> had higher O <sub>2</sub> -affinity at 4 °C	Genetic differences in temperature specific growth rate cannot be overcome by acclimation	Pörtner et al. (2001)

Lab	Growth O <sub>2</sub> -affinity 4, 12 °C <i>In-vitro</i>	1-group	Farmed (origin: W. Norway and Barents Sea)	44 and 16 (O <sub>2</sub> )	No differences Highest O <sub>2</sub> -affinity at 12 °C irrespective of genotype	Temperature drives the aggregation of physiologically similar individuals	Colosimo et al. (2003)
Lab	T <sub>pref</sub>	Juv.	Øresund	16	<i>Hbl-2/2</i> : 8.2 ± 1.5 °C (norm/hypoxia) <i>Hbl-1/1</i> : 15.4 ± 1.1 °C (normoxia) and 9.8 ± 1.8 °C (hypoxia) <i>Hbl-2/2</i> have higher O <sub>2</sub> -affinity at 4 °C No differences at 12 °C	A similar decrease in T <sub>pref</sub> for <i>Hbl-2/2</i> may occur at O <sub>2</sub> levels < 33%	Petersen and Steffensen (2003) Brix et al. (2004)
Lab	O <sub>2</sub> -affinity 4, 12 °C <i>In-vitro</i>	1-group	Farmed (origin: W. Norway)	126	<i>Hbl-2/2</i> have higher O <sub>2</sub> -affinity at 4 °C No differences at 12 °C	Acclimation changes the relative amount of the different Hb components	Brix et al. (2004)
Lab	Growth	Juv.	Farmed 9–11 °C (origin: W. Norway and Barents Sea)	Ca. 6100	No differences		Gjerde et al. (2004)
Lab	Growth 7, 10, 13, 16 °C and T-steps (16, 13, 10 °C)	Juv.	Farmed 10 °C (Origin: Western Norway)	220	<i>Hbl-2/2</i> have higher growth rate at 13–16 °C <i>Hbl-1/1</i> at 7 °C No differences at 12 °C (most pronounced under T-step exp.)	<i>Hbl-1/1</i> have lowest T <sub>opt</sub> (12.5 °C) and <i>Hbl-1/2</i> highest (14.5 °C) Perhaps due to differences in metabolic capacity	Imsland et al. (2004)
Lab	Growth (4–16 °C)	Juv.	Farmed + wild (origin: W. Norway and Barents Sea)	570	No differences	Not able to distinguish between differences related to family group and genotype	Jørstad et al. (2006)
Lab	Growth 6, 14 °C Energy retention and feed conversion efficiency	Juv.	Farmed 8 °C (origin: W. Norway)	306	No differences in growth <i>Hbl-2/2</i> have a tendency for highest energy retention and feed conversion efficiency	T <sub>pref</sub> is not necessarily a good indicator for T <sub>opt</sub>	Jordan et al. (2006)
Lab	Hypoxia avoidance behaviour 5, 15 °C	Juv.	Farmed 8–10 °C (origin: Bergen)	28	No differences in avoidance response or ventilation rates <i>Hbl-1/1</i> swam faster than <i>Hbl-2/2</i> at 15 °C	Overall reduced swimming speed and avoidance of most hypoxic habitat	Skjæraasen et al. (2008)
Lab	Temp. and hypoxia avoidance	Juv.	Farmed 10 °C (origin: Nova Scotia)	100– 400	No significant differences but <i>Hbl-1/1</i> had lower S <sub>crit</sub> indicating that this genotype is slightly more tolerant to hypoxia <i>Hbl-1/1</i> grew faster in the first 9 months and had higher mortalities	Differences in growth is likely not caused by reduced SMR or higher metabolic capacity	Gamperl et al. (2009)
Lab	Hypoxia/stress response 5, 10 and 15 °C	Juv.	Øresund	50–60	<i>Hbl-1/1</i> have higher plasma cortisol and lactate levels indicative of an increased level of stress	<i>Hbl-2/2</i> are perhaps more tolerant to hypoxia	Methling et al. (2010)
Lab	T <sub>pref</sub>	Juv.	Øresund	24	<i>Hbl-2/2</i> : 8.9 ± 0.2 °C <i>Hbl-1/1</i> : 11.0 ± 0.6 °C <i>Hbl-1/2</i> have a wider range of T <sub>pref</sub>	<i>Hbl-1/2</i> are likely more flexible	Behrens et al. (2012)



In most growth studies fish are fed *ad libitum*, simulating commercial fish farm conditions. However, acknowledging that food is rarely found in excess in nature and further that fish behave differently according to their personality (Adriaenssens and Johnsson, 2011), keeping experimental animals under such artificial conditions may likely produce results not comparable to what is observed in the wild. Salvanes and Hart (2000) investigated feeding behaviour in cod with different haemoglobin genotypes and found that *Hbl-2/2* had the highest prey capture success and were generally the first to take prey. It was, hence, concluded that in situations where fish have to compete for food, cod homozygous for *Hbl<sup>2</sup>* have an advantage. Exposing experimental animals to more natural feeding conditions would presumably result in more pronounced behavioural differences, mimicking those seen in nature.

Another issue in relation to behaviour is that genotypes are often held together randomly with no consideration of haemoglobin composition. If a tank is dominated by fish homozygous for *Hbl<sup>2</sup>*, that are known to be more competitive, this is likely to influence the results. Salvanes and Hart (2000) used two types of experimental setup; one in which three fish (one of each genotype) were held together and another in which four fish were chosen at random. The “group” factor was shown to have a significant effect on feeding behaviour, emphasizing the importance of knowing and if necessary manipulating the haemoglobin composition in tanks. Additionally, the experiments should be replicated to control for “tank effects”. In this study *Hbl-2/2* was shown to be superior, however other combinations of haemoglobin genotypes may result in a shift in competitive superiority (Salvanes and Hart, 2000). In nature, cod of different genotypes usually co-exist, and it would, thus, be interesting to test for competitive performance within different groupings. Only in one of the growth studies were the genotypes reared separately (Jørstad et al., 2006) and the results did not show any differences in growth.

Considering that cod show plasticity in their expression of the different haemoglobin components (Brix et al., 2004; Andersen et al., 2009; Borza et al., 2009), an acclimation period prior to temperature experiments will likely influence the growth rate and perhaps impede the actual haemoglobin effects. In addition to this, epigenetic compensation mechanisms controlled by other loci may disguise these effects further (Stearns, 1992; Donelson et al., 2012). The length of the acclimation period varies significantly between studies; from a few days (Jordan et al., 2006; Gamperl et al., 2009) up to several months (Nævdal et al., 1992; Imsland et al., 2004). A long acclimation period seem to reduce the differences between the genotypes, acclimation time should be kept at a minimum. However, this raises the problem that stressed fish most likely will not feed voluntarily, which consequently influences the results. A short acclimation period may, in theory, favour the more stress-resistant *Hbl* type.

In most of the experimental studies, 2nd generation cod i.e., offspring from wild-caught fish, were used. Collection of the parental fish from the wild differed between the studies both with regard to the time of year and fishing method (trawl, long line, etc.). This may have had large impacts on the experiments described previously due to differences in the environment inhabited by the parent fish. Seasonal changes such as migration to and from spawning/feeding areas as well as gear type will influence the “selection of fish”, likely biasing the sampling in a particular, however, largely unknown direction. The conditions experienced in captivity are far from natural, despite attempts to make them so i.e., matching the photoperiod and temperature experienced by the fish in the wild. Lack of predators, limited space for movement and constant feeding regimes constitute some of the main differences. These will inevitably result in some degree of acclimatisation, and this phenotypic plasticity may be reflected in the offspring. Studies, in which offspring from farmed fish have been used, may also be a source of variation as these fish are likely to have adapted to the farmed conditions through several generations.

#### 4. Discussion and conclusion

The observed latitudinal cline in *Hbl<sup>1</sup>* frequency is widely believed to be the result of selection with cod homozygous for *Hbl<sup>1</sup>* and *Hbl<sup>2</sup>* preferring warmer and colder waters, respectively. This relationship is also reflected in the oxygen affinity, which is highest for *Hbl-2/2* at lower temperatures and vice versa for *Hbl-1/1*. The heterozygote is somewhere in-between and is likely to be the most flexible of the three common genotypes as it possesses both haemoglobins in equal quantities.

Despite the clear association between the haemoglobin genotype and the preferred temperature and oxygen affinity, correlations with other physiological traits such as thermal tolerance and growth are less obvious. This may be due to both the difficulty of estimating the influence of a single factor (*Hbl*) when a number of factors interact, and also to limitations of experimental designs.

Fish growth is affected by multiple environmental and genetic factors. Laboratory experiments allow for a certain amount of control and reduce some of the influential factors but, nevertheless, introduce new problems such as tank and sibling effects. Experimenters also have to deal with fish personality (competition, aggression, and cooperative behaviour), acclimation time and feeding regime which are all likely to affect the outcome, although often in unpredictable ways.

Having emphasised all the problems and pitfalls with different experimental setups, it seems appropriate to outline what a good approach to study *Hbl* genotypic growth rates is. Experiments should be performed on full-siblings by crossing of two wild-caught heterozygotes. Several families should be included in the study allowing for differences in phenotypic plasticity due to variation in the environment inhabited by the families (northern/southern latitudes, different depth regimes, coastal/offshore, etc.). Full-siblings within each family should be exposed to different temperature regimes and oxygen saturation levels, and if possible also different feeding regimes (*ad libitum*/minimum). The tanks should ideally be deep, allowing for vertical migrations, and the haemoglobin composition should either be balanced or differ between tanks to test for effects of competition (and fish behaviour in general). Acclimation periods should be kept at a minimum, yet enabling all the fish to fully recover from stress, to allow for the actual *Hbl* genotypic variation to manifest itself. Fish farm facilities would probably be the most suitable for such a large-scale experiment which is, of course, very labour- and costs consuming.

An enhanced understanding of cod haemoglobins and their influence on various physiological traits will help both in understanding genome–environment interactions in natural cod populations, and be beneficial for optimizing the conditions for cod rearing in aquaculture. The knowledge will give better insight into processes affected by climate change, making prediction of the response of cod with different haemoglobin genotypes more reliable. In aquaculture, the knowledge can be used to improve facility settings to fit a particular *Hbl* genotype, potentially maximizing the yield.

The candidate gene approach (single nucleotide polymorphisms, SNPs) may offer some promising prospects in relation to our understanding of natural selection working on specific genes and resulting in certain life-history traits and strategies (Hemmer-Hansen et al., 2011). Polymorphic candidate genes of known function can link genetic variation with individual differences in physiological functions (Hemmer-Hansen et al., 2011). For example, an association between particular SNPs and growth has been demonstrated in Arctic charr (*Salvelinus alpinus*) (Tao and Boulding, 2003). Efforts to relate genetic variation with physiological traits using SNPs have also been made for Atlantic cod (Moen et al., 2008; Nielsen et al., 2009; Hubert et al., 2010). However, whether the candidate gene approach can shed light on the effects of different haemoglobins and potential cooperation between haemoglobin loci is yet to be investigated.

In conclusion, it is important to keep in mind that haemoglobin is not the only factor influencing the physiology of cod – epistatic interactions as well as pleiotrophic effects are likely to be involved in shaping the various phenotypes observed in different experimental situations. To establish an experimental design, which can disentangle the various genetic sources of variation and their interactions is a very challenging goal, thus the underlying complexities must be kept in mind when interpreting experimental results.

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## References

- Adriaenssens, B., Johnsson, J.I., 2011. Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. *Behav. Ecol.* 22 (1), 135–143.
- Andersen, Ø., 2012. Hemoglobin polymorphisms in Atlantic cod – a review of 50 years of study. *Mar. Genomics* 8, 59–65.
- Andersen, Ø., Wetten, O.F., De Rosa, M.C., Andre, C., Alinovi, C.C., Colafranceschi, M., Brix, O., Colosimo, A., 2009. Haemoglobin polymorphisms affect the oxygen-binding properties in Atlantic cod populations. *Proc. R. Soc. B Biol. Sci.* 276, 833–841.
- Behrens, J., Gråns, A., Thorkildsen, N.O., Neuenfeldt, S., Axelsson, M., 2012. Correlations between haemoglobin type and temperature preference of juvenile Atlantic cod *Gadus morhua*. *J. Exp. Mar. Biol. Ecol.* 413, 71–77.
- Björnsson, B., Steinarsson, A., 2002. The food-unlimited growth rate of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 59, 494–502.
- Borza, T., Stone, C., Gamperl, A.K., Bowman, S., 2009. Atlantic cod (*Gadus morhua*) hemoglobin genes: multiplicity and polymorphism. *BMC Genet.* 10, 51.
- Brett, J.R., 1971. Energetic responses of salmon to temperature. A study of some thermal regulations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *Am. Zool.* 11, 99–113.
- Brix, O., Forås, E., Strand, I., 1998. Genetic variation and functional properties of Atlantic cod hemoglobins: introducing a modified tonometric method for studying fragile hemoglobins. *Comp. Biochem. Physiol.* A 119, 575–583.
- Brix, O., Thorkildsen, S., Colosimo, A., 2004. Temperature acclimation modulates the oxygen binding properties of the Atlantic cod (*Gadus morhua* L.) genotypes Hbl\*1/1, Hbl\*1/2, and Hbl\*2/2 by changing the concentrations of their major hemoglobin components (results from growth studies at different temperatures). *Comp. Biochem. Physiol.* A 138, 241–251.
- Buhler, D.R., Shanks, W.E., 1959. Multiple hemoglobins in fishes. *Science* 129 (3353), 899–900.
- Claireaux, G., Webber, D.M., Lagardere, J.P., Kerr, S.R., 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). *J. Sea Res.* 44, 257–265.
- Colosimo, A., Giuliani, A., Maranghi, F., Brix, O., Thorkildsen, S., Fischer, T., Knust, R., Pörtner, H.O., 2003. Physiological and genetical adaptation to temperature in fish populations. *Cont. Shelf Res.* 23, 1919–1928.
- Dahle, G., Jørstad, K.E., 1993. Haemoglobin variation in cod – a reliable marker for Arctic cod (*Gadus morhua* L.). *Fish. Res.* 16, 301–311.
- Dahle, G., Jørstad, K.E., Rusaas, H.E., Otterå, H., 2006. Genetic characteristics of broodstock collected from four Norwegian coastal cod (*Gadus morhua*) populations. *ICES J. Mar. Sci.* 63, 209–215.
- Donelson, J.M., Munday, P.L., McCormick, M.I., Pitcher, C.R., 2012. Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nat. Clim. Chang.* 2, 30–32.
- Dutil, J.D., Brander, K.M., 2003. Comparing productivity of North Atlantic cod (*Gadus morhua*) stocks and limits to growth production. *Fish. Oceanogr.* 12, 502–512.
- Frydenberg, O., Møller, D., Nævdal, G., Sick, K., 1965. Haemoglobin polymorphism in Norwegian cod populations. *Hereditas-Genetisk A.* 53, 257–271.
- Fyhn, U.E.H., Brix, O., Nævdal, G., Johansen, T., 1994. New variants of the haemoglobins of Atlantic cod – a tool for discriminating between coastal and Arctic cod populations. *Int. Co. Explor. Sea, Copenhagen K.*
- Fyhn, U.E.H., Johansen, T., Nævdal, G., Thorkildsen, S., 1995. Ontogeny of haemoglobin polymorphism in cod. *J. Fish Biol.* 47, 56–60.
- Gamperl, A.K., Busby, C.D., Hori, T.S.F., Afonso, L.O.B., Hall, J.R., 2009. Hemoglobin genotype has minimal influence on the physiological response of juvenile Atlantic cod (*Gadus morhua*) to environmental challenges. *Physiol. Biochem. Zool.* 82, 483–494.
- Gjerde, B., Terjesen, B.F., Barr, Y., Lein, I., Thorland, I., 2004. Genetic variation for juvenile growth and survival in Atlantic cod (*Gadus morhua*). *Aquaculture* 236, 167–177.
- Gjøsaeter, J., Jørstad, K., Nævdal, G., Thorkildsen, S., 1992. Genotype distributions of cod from the Norwegian Skagerrak coast. *Sarsia* 76, 255–259.
- Glover, K., Nyhammer, G., Nævdal, G., Otterlei, E., Thorkildsen, S., 1997. Studies on genotype dependent growth in juvenile cod (*Gadus morhua*) reared at different temperatures and light regimes. Report Series, 1997 (8). Department of Fisheries and Marine Biology, University of Bergen, Norway (13 pp.).
- Godø, O.R., Moksness, E., 1987. Growth and maturation of Norwegian coastal cod and Northeast Arctic cod under different conditions. *Fish. Res.* 5 (2–3), 235–242.
- Gråns, A., Olsson, C., Pitsillides, K., Nelson, K.H.E., Cech Jr., J.J., Axelsson, M., 2010. Effects of feeding on thermoregulatory behaviours and gut blood flow in white sturgeon (*Acipenser transmontanus*) using biotelemetry in combination with standard techniques. *J. Exp. Biol.* 213, 3198–3206.
- Halldórsdóttir, K., Árnason, E., 2009a. Multiple linked  $\beta$  and  $\alpha$  globin genes in Atlantic cod: a PCR based strategy of genomic exploration. *Mar. Genomics* 2, 169–181.
- Halldórsdóttir, K., Árnason, E., 2009b. Organization of a  $\beta$  and  $\alpha$  globin gene set in the teleost Atlantic cod, *Gadus morhua*. *Biochem. Genet.* 47, 817–830.
- Hemmer-Hansen, J., Nielsen, E.E., Meldrup, D., Mittelholzer, C., 2011. Identification of single nucleotide polymorphisms in candidate genes for growth and reproduction in a nonmodel organism: the Atlantic cod *Gadus morhua*. *Mol. Ecol. Resour.* 11 (Suppl. 1), 71–80.
- Hubert, S., Higgins, B., Borza, T., Bowman, S., 2010. Development of a SNP resource and a genetic linkage map for Atlantic cod (*Gadus morhua*). *BMC Genomics* 11, 191.
- Husebø, A., Imsland, A.K., Nævdal, G., 2004. Haemoglobin variation in cod: a description of new variants and their geographical distribution. *Sarsia* 89, 369–378.
- Hysten, A., 1964. Coastal cod and skrei in the Lofoten area. *FiskDir. Skr. Ser. HavUnders.* 13, 27–42.
- ICES, a. [www.ices.dk](http://www.ices.dk).
- Imsland, A.K., Foss, A., Nævdal, G., Johansen, T., Folkvord, A., Stefansson, S.O., Jonassen, T.M., 2004. Variations in growth in haemoglobin genotypes of Atlantic cod. *Fish Physiol. Biochem.* 30, 47–55.
- Jamieson, A., Birley, A.J., 1989. The demography of a haemoglobin polymorphism in the Atlantic cod, *Gadus morhua* L. *J. Fish Biol.* 35 (Suppl. A), 193–204.
- Jamieson, A., Jónsson, J., 1971. The Greenland component of spawning cod at Iceland. *Rapports et Procès-Verbaux des Réunions. Cons. Int. Explor. Mer* 161, 65–72.
- Jamieson, A., Otterlei, G., 1971. The use of cod blood protein polymorphisms in the Belt Sea, the Sound and the Baltic Sea. *Rapports et Procès-Verbaux des Réunions. Cons. Int. Explor. Mer.* 161, 55–59.
- Jamieson, A., Thompson, D., 1972. Blood proteins in North Sea cod (*Gadus morhua* L.). XIIIth European Conference on Animal Blood Groups Biochemical Polymorphism, Bp. 585–591.
- Jensen, F.B., Fago, A., Weber, R.E., 1998. Hemoglobin structure and function. *Fish Physiol.* 17, 1–40.
- Jobling, M., 1983. Growth-studies with fish – overcoming the problems of size variation. *J. Fish Biol.* 22, 153–157.
- Jordan, A.D., Lampe, J.F., Grisdale-Helland, B., Helland, S.J., Shearer, K.D., Steffensen, J.F., 2006. Growth of Atlantic cod (*Gadus morhua* L.) with different haemoglobin subtypes when kept near their temperature preference. *Aquaculture* 257, 44–52.
- Jørstad, K.E., Nævdal, G., 1994. Studies on associations between genotypes and growth rate in juvenile cod. *ICES Mar. Sci.* 198, 671–675.
- Jørstad, K.E., Karlsen, O., Svasand, T., Otterå, H., 2006. Comparison of growth rate among different protein genotypes in Atlantic cod, *Gadus morhua*, under farmed conditions. *ICES J. Mar. Sci.* 63 (2), 235–245.
- Karpov, A.K., Novikov, G.G., 1980. Hemoglobin alloforms in the cod *Gadus morhua*, gadiformes Gadidae. Their functional features and distribution in populations. *J. Ichthyol.* 20 (6), 45–49.
- Karpov, A.K., Osinov, A.G., Novikov, G.G., 1984. Protein polymorphism in Atlantic cod, *Gadus morhua* (Gadidae), of the White Sea. *J. Ichthyol.* 24, 71–79.
- Kirpichnikov, V.S., 1981. Genetic Bases of Fish Selection. Springer-Verlag, Berlin (410 pp.).
- Lefrançois, C., Claireaux, G., 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*. *Mar. Ecol. Prog. Ser.* 259, 273–284.
- McFarland, S., 1998. Biochemical and Physiological Adaptations of Haemoglobin-I Genotypes of Atlantic Cod, *Gadus morhua* L. The University of Birmingham (PhD thesis).
- Methling, C., Aluru, N., Vijayan, M.M., Steffensen, J.F., 2010. Effect of moderate hypoxia at three acclimation temperatures on stress responses in Atlantic cod with different haemoglobin types. *Comp. Biochem. Physiol.* A 156, 485–490.
- Moen, T., Hayes, B., Nilsen, F., Delghandi, M., Fjalestad, K.T., Fevolden, S.E., Berg, P.R., Lien, S., 2008. Identification and characterisation of novel SNP markers in Atlantic cod: evidence for directional selection. *BMC Genet.* 9, 18.
- Møller, D., 1966. Genetic differences between cod groups in the Lofoten area. *Nature* 19, 824.
- Møller, D., 1968. Genetic diversity in spawning cod along the Norwegian coast. *Hereditas* 60, 1–32.
- Mork, J., Sundnes, G., 1984. Hemoglobin polymorphism in *Gadus Morhua*: genotypic differences in hematocrit. *Helgol. Meeresunt.* 38, 201–206.
- Mork, J., Sundnes, G., 1985. Hemoglobin polymorphism in Atlantic cod (*Gadus morhua*): allele frequency variation between year classes in a Norwegian fjord stock. *Helgol. Meeresunt.* 39, 55–62.
- Mork, J., Giskeødegard, R., Sundnes, G., 1983. The haemoglobin polymorphism in *Gadus morhua*: genotypic differences in maturing age and within-season gonad maturation. *Helgol. Meeresunt.* 36, 313–322.
- Mork, J., Giskeødegard, R., Sundnes, G., 1984a. The haemoglobin polymorphism in Atlantic cod (*Gadus morhua* L.): genotypic differences in somatic growth and in maturing age in natural populations. *Flødevigen Rapportserie* 1, 721–732.
- Mork, J., Giskeødegard, R., Sundnes, G., 1984b. Population genetic studies in cod (*Gadus morhua* L.) by means of the haemoglobin polymorphism: observations in a Norwegian coastal population. *FiskDir. Skr. (Havundersøgelser)* 17, 449–471.
- Mork, J., Ryman, N., Ståhl, G., Utter, F., Sundnes, G., 1985. Genetic variation in Atlantic cod (*Gadus morhua*) throughout its range. *Can. J. Fish. Aquat. Sci.* 42, 1580–1587.
- Nævdal, G., Folkvord, A., Otterlei, E., Thorkildsen, S., 1992. Growth-rate related to genotype of 0-group cod at 3 environmental temperatures. *Sarsia* 77, 71–73.
- Nielsen, E.E., Hemmer-Hansen, J., Poulsen, N.A., Loeschke, V., Moen, T., Johansen, T., Mittelholzer, C., Taranger, G.L., Ogden, R., Carvalho, G.R., 2009. Genomic signatures

- of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). *BMC Evol. Biol.* 9, 276.
- Nikinmaa, M., 2001. Haemoglobin function in vertebrates: evolutionary changes in cellular regulation in hypoxia. *Resp. Physiol.* 128 (3), 317–329.
- Nordeide, J.T., Båmstedt, U., 1998. Coastal cod and north-east Arctic cod – do they mingle at the spawning grounds in Lofoten? *Sarsia* 83 (5), 373–379.
- Pampoulie, C., Ruzzante, D.E., Chosson, V., Jörundsdóttir, T.D., Taylor, L., Thorsteinsson, V., Daniélsdóttir, A.K., Marteinsdóttir, G., 2011. The genetic structure of Atlantic cod (*Gadus morhua*) around Iceland: insight from microsatellites, the Pan I locus, and tagging experiments. *Can. J. Fish. Aquat. Sci.* 63 (12), 2660–2674.
- Pedersen, T., Jobling, M., 1989. Growth rates of large, sexually mature cod, *Gadus morhua*, in relation to condition and temperature during an annual cycle. *Aquaculture* 81, 161–168.
- Petersen, M.F., Steffensen, J.F., 2003. Preferred temperature of juvenile Atlantic cod *Gadus morhua* with different haemoglobin genotypes at normoxia and moderate hypoxia. *J. Exp. Biol.* 206, 359–364.
- Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315, 95–97.
- Pörtner, H.O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T., Knust, R., Lannig, G., Naevdal, G., Nedenes, A., Nyhammer, G., Sartoris, F.J., Serendero, I., Sirabella, P., Thorkildsen, S., Zakhartsev, M., 2001. Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). *Cont. Shelf Res.* 21, 1975–1997.
- Rätz, H.J., Lloret, J., 2003. Variation in fish condition between Atlantic cod (*Gadus morhua*) stocks, the effect on their productivity and management implications. *Fish. Res.* 60, 369–380.
- Righton, D.A., Andersen, K.H., Neat, F., Thorsteinsson, P.S., Svedäng, H., Michalsen, K., Hinrichsen, H.H., Bendall, V., Neuenfeldt, S., Wright, P., Jonsson, P., Huse, G., van der Kooij, J., Mosegaard, H., Hüsey, K., Metcalfe, J., 2010. Thermal niche of Atlantic cod *Gadus morhua*: limits, tolerance and optima. *Mar. Ecol. Prog. Ser.* 420, 1–13.
- Rollefsen, G., 1934. The cod otolith as a guide to race, sexual development and mortality. *Rapp. P.V. Reun. Cons. Int. Explor. Mer.* 88, 1–15.
- Salvanes, A.G.V., Hart, P.J.B., 2000. Is individual variation in competitive performance of reared juvenile cod influenced by haemoglobin genotype? *Sarsia* 85, 265–274.
- Schurmann, H., Steffensen, J.F., 1992. Lethal oxygen levels at different temperatures and the preferred temperature during hypoxia of the Atlantic cod *Gadus morhua* L. *J. Fish Biol.* 41, 927–934.
- Sick, K., 1961. Haemoglobin polymorphism in fishes. *Nature* 192, 894–896.
- Sick, K., 1965a. Haemoglobin polymorphism of cod in Baltic and Danish Belt Sea. *Hereditas-Genetisk A.* 54, 19–48.
- Sick, K., 1965b. Haemoglobin polymorphism of cod in North Sea and North Atlantic Ocean. *Hereditas-Genetisk A.* 54, 49–69.
- Skjæraasen, J.E., Nilsen, T., Meager, J.J., Herbert, N.A., Moberg, O., Tronci, V., Johansen, T., Salvanes, A.G.V., 2008. Hypoxic avoidance behaviour in cod (*Gadus morhua* L.): the effect of temperature and haemoglobin genotype. *J. Exp. Mar. Biol. Ecol.* 358, 70–77.
- Stearns, S.C., 1992. *The Evolution of Life Histories*. University Press, Oxford (248 p.).
- Swain, D.P., Kramer, D.L., 1995. Annual variation in temperature selection by Atlantic cod *Gadus morhua* in the southern Gulf of St. Lawrence, Canada, and its relation to population size. *Mar. Ecol. Prog. Ser.* 116, 11–23.
- Tao, W.J., Boulding, E.G., 2003. Associations between single nucleotide polymorphisms in candidate genes and growth rate in Arctic charr (*Salvelinus alpinus* L.). *Heredity* 91 (1), 60–69.
- Taranger, G.L., Aardal, L., Hansen, T., Kjesbu, O.S., 2006. Continuous light delays sexual maturation and increases growth of Atlantic cod (*Gadus morhua* L.) in sea cages. *ICES J. Mar. Sci.* 63 (2), 365–375.
- Val, A.L., 2000. Organic phosphates in the red blood cells of fish. *Comp. Biochem. Physiol.* A 125, 417–435.
- Verde, C., Balestrieri, M., Pascale, D.M., Pagnozzi, D., Lecointre, G., Prisco, G., 2006. The oxygen transport system in three species of the boreal fish family Gadidae. *J. Biol. Chem.* 28, 22073–22084.
- Weber, R.E., Jensen, F.B., 1988. Functional adaptations in hemoglobins from ectothermic animals. *Annu. Rev. Physiol.* 50, 161–179.
- Wennevik, V., Jørstad, K.E., Dahle, G., Fevolden, S.-E., 2008. Mixed stock analysis and the power of different classes of molecular markers in discriminating coastal and oceanic Atlantic cod (*Gadus morhua* L.) on the Lofoten spawning grounds, Northern Norway. *Hydrobiologia* 606, 7–25.
- Wilkins, N.P., 1967. Polymorphism of whole blood proteins in the cod (*Gadus morhua* L.). *Cons. Perm. Int. Expl. Mer.* 31 (1), 77–88.
- Wilkins, N.P., 1969. Haemoglobin polymorphism in cod, whiting and Pollack in Scottish waters. *Cons. Perm. Int. Explor. Mer.* 161, 60–63.