Development of genomics tools and elite broodstock for Atlantic cod culture in Canada

Edward A. Trippel¹, Matthew L. Rise², A. Kurt Gamperl², Stewart C. Johnson³, J. Andy B. Robinson⁴, Keith Culver⁵,⁶, Marlies Rise² and Sharen Bowman⁷

In recent years, there has been interest in the development of Atlantic cod (Gadus morhua) mariculture in North America, Norway, the UK, the Faroe Islands and Iceland (Kjesbu et al. 2006). This arose mainly because depressed cod stocks, such as those in eastern Canada and the northeastern United States, showed little sign of recovery and this provided an opportunity for cultured cod to augment wild cod in traditional or newly developed markets.

Cod culture in Canada began in the 1980s in Newfoundland and was routinely based on gametes collected from wild broodstock (F0) or the growout of wild caught juveniles known as cod ranching (Brown et al. 2003, Kjesbu et al. 2006). With the cod moratorium in eastern Canada beginning in 1992, cod culture techniques to consistently produce juveniles for farming were developed in Newfoundland and elsewhere (Brown et al. 2003). In addition, a shift in gadoid species research occurred from haddock (Melanogrammus aeglefinus) to cod in New Brunswick in 2004, due in part to a change in industrial partners and the observed poor growth rate of cage-reared haddock (Frantsi et al. 2002, Bjørnsson et al. 2010). Within this context and, given the regional and national importance of aquaculture, the $18.1 million Atlantic Cod Genomics and Broodstock Development Project (CGP; www.codgene.ca) was funded in part by Genome Canada, Genome Atlantic and the Atlantic Canada Opportunities Agency (ACOA) in 2006, with the objective of developing a cod breeding program and a set of genomics tools in conjunction with industry to accelerate the development of Atlantic cod aquaculture in Atlantic Canada (Figure 1). This article briefly summarizes the structure of the CGP, its significance to cod culture and key achievements over the CGPs 4.5 year duration, and forecasts its legacy.

Selective Breeding

Cod were reared from egg to juvenile stage at the St. Andrews Biological Station (Fisheries and Oceans Canada) and Huntsman Marine Science Centre in New Brunswick, at GreatBay Aquaculture Inc. in New Hampshire, and at the Dr. Joe Brown Aquatic Research Building (JBARB) of the Ocean Sciences Centre (Memorial University of Newfoundland). The hatcheries at each facility housed broodstock of local origin, thereby serving the development of two key centers of cod farming in Atlantic Canada, one in the Bay of Fundy and the other in Newfoundland. We designed the breeding program to explore the relative merits of two different approaches to hatching rearing of families for performance evaluation. In the first, cod families were raised in separate 500 L tanks (one per family) until they reached a suitable size for PIT (passive integrated transponder) tagging (~15 g), and in this way, no financial cost for genotyping was incurred to identify family at harvest sampling (Figure 2). Using this method, a total of 51,547 juveniles were produced at the various production sites. Although a breeding design that incorporated half-sib families and statistical analyses partly accounted for tank effects associated with single family rearing, a family’s performance may still be influenced by tank effects, such as different fish densities, cannibalism, prey density and oxygen levels. To explore this further, we also established 8-10 communal family rearing tanks (3,000 L) annually in New Brunswick, each of which was stocked with 15-20 families of recently hatched larvae, and these were grown to 30-60 g before being sent to the sea-cages. In the autumn of each year, juveniles from the two rearing approaches that

Fig. 1. Atlantic cod (Gadus morhua) raised in captivity as part of the Cod Genomics Project.
shared the same parents were co-stocked into sea cages (Figure 3) and tissue samples of untagged fish were collected at harvest and genotyped to assign family.

There were four spawning seasons (2006-2009) and, in total, this resulted in the stocking in sea cages of 168,019 juveniles, including PIT tagged fish, as well as fish from families that were pooled and not PIT tagged, in New Brunswick and 15,069 juveniles, PIT tagged fish only, in Newfoundland for family evaluation. Six hundred twelve families were generated over four years, and partly in the third year and fully in the fourth year these were F2 offspring of elite F1 selected parents. An excess 192,185 juvenile cod were also produced and transferred to sea cages for growout by our industry partners; Cooke Aquaculture Inc. in New Brunswick, and Northern Cod Ventures and Newfoundland Cod Broodstock Company in Newfoundland.

Valuable traits, such as growth, immune function/disease resistance, the presence of deformities, sexual maturation, stress tolerance, fillet quality and yield were evaluated at the two locations (~2 years post-stocking). When possible, we compared family growth performance using fish that were reared as individual families and communally. High heritability estimates were reported for growth (size at harvest; $h^2 \sim 0.45$; Tosh et al. 2010, Garber et al. 2010), and this was the key trait used to determine Estimated Breeding Values (EBV). These EBVs were used to guide the selection of elite families with projected superior offspring quality to act as broodstock, and those fish were brought back from the sea cages to the land-based facilities in each province. At the Ocean Sciences Centre, 5,830 cod were also reared from the embryo stage in captivity for multiple years to safeguard against storms, accidental or disease-related losses in the sea cages. This allowed us to have a reserve source of elite broodstock and provided an additional opportunity to evaluate family performance in both the laboratory and sea cages.

Photoperiod manipulation of broodstock was used in Newfoundland to advance ovulation by six months. This allowed for the stocking of juveniles into sea cages in the spring, and thus, for the fish to take advantage of favorable summer water temperatures during their first year. Reproductive studies in New Brunswick led to the development of techniques for the induction of spawning to aid in the stripping of gametes and for the cryopreservation of sperm samples (Garber et al. 2009, Butts et al. 2010). Sperm cryopreservation can be used to augment breeding and to maintain the male genome of elite families in perpetuity.

**Genomics**

Prior to the CGP, Atlantic cod were underrepresented in public sequence databases, with only 2,776 sequences available for this species. The CGP addressed this issue by generating a large number of expressed sequence tags (ESTs; Bowman et al. In Press). These are short (~500-600 base pair long on average) sections of transcribed sequences, the vast majority of which represent protein coding genes. Approximately 158,000 EST sequences were deposited in GenBank during the course of the CGP, and this project remains the major contributor of Atlantic cod sequences to public databases to date. The ESTs generated were also clustered to allow for the identification of unique transcripts. In total, 51,814 unique sequences were identified. However, this is likely to be an overestimate of the total number of transcripts as different, non-overlapping, regions may have been sequenced in some cases.

The ESTs generated were used in several analyses. Initially, the set of unique sequences was compared to sequences present in the public databanks for other species. This allowed us to identify similar sequences, and thus, to facilitate annotation of the Atlantic cod sequence collection. In total, 15,873 (31 percent) of the set of unique sequences had a significant hit in the public databases (Bowman et al. In Press). This information, together with the results of addi-
Fig. 4. Genomics tools development and applications of the Cod Genomics Project.

tional analyses, is available from the project database (http://ri.imb.nrc.ca/codgene).

As the CGP sequences were generated from several hundred individual fish, we also examined our sequence collection to identify genetic variation, both in the form of microsatellite sequences (short, repetitive regions; Higgins et al. 2009) and single nucleotide polymorphisms (SNPs: variation at a single base; Hubert et al. 2010). After testing, the EST collection was isolated from fish enrolled in the CGP breeding programs, thus, we anticipated that the markers generated would be informative within those programs. This proved to be the case and we were successful in identifying SNPs associated with QTLs for most of the traits under study. We were also able to generate a high density genetic linkage map for Atlantic cod using a small number of mapping families that were genotyped with our SNP set (Hubert et al. 2010).

In addition to identifying SNPs, the ESTs generated by the CGP were used to construct a 20,000 element (20K) oligonucleotide microarray for use in expression analyses (Booman et al. In Press). Sequences used in the construction of the CGP 20K microarray were selected from the set of unique sequences based on the following criteria: a) those with similarity to an entry in the public databases were prioritized; and b) sequences with no similarities if they were represented multiple times within the CGP dataset or if they were highly represented in cDNA libraries generated using suppression subtractive hybridization (SSH; Diatchenko et al. 1996). The latter is a technique that can be used to identify transcripts up- or down-regulated by applied stimuli such as immunogenic agents or a temperature challenge (Rise et al. 2008, 2010, Feng et al. 2009, Hori et al. 2010). The CGP 20K microarray has been constructed and successfully tested, and is currently being applied to investigate the global gene expression responses of Atlantic cod to bacterial, viral and temperature challenges.

The CGP has generated as part of its legacy an extensive set of genomic tools for Atlantic cod (Figure 4). These include the EST sequences and SNP markers described here, which have been deposited in the public databases and are available for all to use, and a 20K oligonucleotide microarray. These resources should enable future research on both wild and cultured Atlantic cod.

Genetic Improvement

Elite broodstock development requires fish rearing and management, and accurate estimates of the genetic merit of superior fish. The components of CGP related to genetic improvement were two-fold: a) the development of statistical genomics tools to produce Estimated Breeding Values (EBVs) and b) an analysis of associations between molecular genetic markers (QTLs) and phenotypes of interest. Statistical genomics tools were developed to assess growth and carcass characteristics (traits) in live fish. When computed with a technique known as best linear unbiased prediction, EBVs account for all known ancestor information and the performance of relatives, offspring and descendents in the determination of the genetic merit of individuals and their suitability for use as broodstock. As part of this process, the
heritability of each trait was computed to determine the degree of inheritance of the characteristic and the resulting potential for genetic improvement.

Along with the above selection tools, marker assisted selection (MAS) offers a valuable approach for broodstock selection. MAS is useful for traits that are difficult to measure, exhibit low heritability, require sacrifice of the animal to measure, and/or are expressed late in development, for example age at sexual maturity. Fish were genotyped for a series of 1,100 SNPs and those genotypes were studied to assess the association between individual SNP marker alleles and superior phenotypes. Developed in this way and used in combination with the EBVs, the markers provide a “handle” to identify the fish with the best combination of genes along with the best EBVs to produce superior broodstock. This technique uses existing variation within the fish to identify superior individuals. The culmination of the genetic improvement component of CGP will be the identification of the optimal combination of EBVs and SNP genotypes to be used in a marker assisted selection program for creating superior broodstock.

**Physiology and Immune Function/Disease Resistance**

CGP research activities in relation to stress include: 1) determining the best parameters to assess stress in cod and determining at which point acute and chronic increases in temperature become stressful; 2) assessing variability in the stress (cortisol) response between families and its association with production relevant traits; 3) determining the number of hemoglobin isoforms in cod, how their expression changes with development and the importance of hemoglobin isoforms to the thermal and hypoxia tolerance of cod; and 4) conducting large-scale gene discovery and expression studies to identify genes involved in the physiological and cellular responses to factors such as acute increases in temperature (Pérez-Casanova *et al.* 2008, Gamperl *et al.* 2009, Hori *et al.* 2010). Finally, considerable variation among cod families with respect to their tolerance to elevated temperatures and stress has been identified. This suggests that there is good potential to selectively breed Atlantic cod for optimal performance in net pens.

With the increased focus on Atlantic cod culture, it has become evident that there are several diseases that may severely limit the success of the industry. CGP research in this area focused on three pathogens: a virus (Atlantic cod nervous necrosis virus; nodavirus), a bacterium (atypical *Aeromonas salmonicida* ) and a microsporidian parasite (*Loma morhua*). These pathogens have been responsible for disease outbreaks in farmed cod populations (*Johnson et al.* 2002, Gagné *et al.* 2004, Samuelsen *et al.* 2006). We are identifying key genes and molecular pathways involved in the innate and adaptive immune responses of Atlantic cod to these pathogens (e.g. see Rise *et al.* 2008, Feng *et al.* 2009, Rise *et al.* 2010) and examining how the expression of these genes differ between fish classified as resistant and susceptible to the diseases these pathogens cause. As with the stress response, a considerable amount of variation among cod families in their resistance to disease has been identified, suggesting that selective breeding may result in more disease resistant Atlantic cod for the industry.

Overall, our research goals in this area were threefold: 1) to identify fish that are disease resistant for use in selective breeding programs; 2) to understand the role that environmental stressors play in the switch from the carrier to disease state; and 3) to utilize the newly developed 20K cod microarray to study the iterative effects of environmental conditions, stress and pathogen exposure on global gene expression responses in immune relevant tissues. The analysis of the results from such an experiment are currently underway, and will provide novel information on how the cod immune system and thus, disease resistance, are likely to be influenced under sea-cage conditions.

**Social Elements**

The CGP also includes a focus on ethical, economic, environmental, legal and social issues associated with the science of genomics. Examples of achievements in this area include the development of solution-oriented legal and policy options regarding legal ownership of commercially valuable research results, the status of elite cod broodstock under Canadian environmental law and Canada’s international obligations, and ethical and legal options regarding benefit sharing and improved methods of consultation with the affected public (*Craik et al.* 2007, Culver 2008).

**Future Directions**

The completion of the CGP has provided the Canadian cod aquaculture industry with elite F2 broodstock and the capacity for marker assisted selection (using QTLs) to further enhance broodstock quality. Over the past four and a half years the CGP engaged academics, post-doctoral fellows, graduate students and government researchers, supported by a well-trained group of technical personnel, with the goals of providing the industry with cod possessing improved growth and survivorship and of developing genomics tools for selecting elite broodstock. Future research will need to target areas that lead to a lowering of production costs. This is because the abundance of some wild cod stocks is highly variable and this has a significant influence on market price. For example, a healthy cod stock presently exists in the Barents Sea and this has placed pressure on Norwegian cod farmers to sell their product at prices below production costs. In addition, the Canadian aquaculture industry is hesitant to broaden its investment in juvenile cod production in the face of the current low market prices, even though Atlantic cod has joined Atlantic salmon (*Salmo salar*) as an endangered species in Canada. The legacy of CGP thus, is the genomics platform/tools developed, which when market conditions improve, can be used to rapidly commercialize cod production. In the meantime, specific problem areas require attention by researchers, including suppression of early puberty, broodstock nutrition and disease susceptibility. They should be addressed using a holistic strategy that, where applicable, integrates genomic-based solutions.
Acknowledgment

This research was supported in part by Genome Canada, Genome Atlantic and the Atlantic Canada Opportunities Agency through the Atlantic Cod Genomics and Broodstock Development Project. A complete list of supporting partners of the Atlantic Cod Genomics and Broodstock Development Project can be found at www.codgene.ca/partners.php. A number of individuals have played important roles in this project, all of which could not be included in authorship. Included are J. Symonds, M. Booman, T. Borza, A. Garber, S. Hubert, J. Tosh, L. Afonso, D. Boyce, D. Hamoutene, L. Lush, S. Neil, J. Symonds, I. Butts, M. Litvak, C. Feng, T. Horii, J. Pérez-Casanova, C. Busby, G. Nash, E. Shine, J. Hall, B. Higgins, J. Kimball, G. Simpson, C. Stone, J. Tarrant Bussey, J. Elliot, J. Moir, G. Nardi, F. Powell, A. Walsh, S. Walker, K. Were, and S. King. Additionally, we greatly appreciate the excellent technical support received from a large number of individuals in laboratories, hatcheries and at cage sites that helped make this project a success.

Notes

1. Fisheries and Oceans Canada, St. Andrews Biological Station, St. Andrews, New Brunswick, Canada.
2. Ocean Sciences Centre, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada.
3. Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, British Columbia, Canada.
4. Centre of Genetic Improvement of Livestock, University of Guelph, Guelph, Ontario, Canada.
5. University of New Brunswick, Fredericton, New Brunswick, Canada.

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