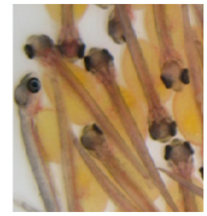
# Egg quality in fish: Present and future challenges



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## Implications

- Egg quality can be defined as the egg ability to be fertilized and subsequently develop into a normal embryo.
- The ability to produce large numbers of high quality eggs "on demand" is an important issue for the development of aquaculture.
- Egg quality is deeply influenced by environmental factors and husbandry practices.
- Egg quality is a difficult to measure phenotype and is therefore not currently included in most selection programs.
- Automatic phenotyping of early embryonic success (i.e., mortalities and deformities) is a challenge.
- Our understanding of what makes a good egg remains incomplete despite the identification of key molecular mechanisms participating in egg quality.
- The identification of robust and generic (i.e., shared by evolutionary distant species) molecular markers of egg quality is a challenge for the future of aquaculture.

**Key words:** aquaculture, broodstock management, maternal effect, molecular markers, phenotyping, teleost

## Introduction

Egg quality or oocyte developmental competence can be defined as the ability of the egg to be fertilized and subsequently develop into a normal embryo (Bobe and Labbé, 2010). Similar definitions have been given by other investigators (Kjörsvik et al., 1990; Brooks et al., 1997) to stress that characterization of several features of early developmental success such as embryo survival or the lack of abnormal development is needed to assess egg quality.

In aquaculture, as in many other animal breeding programs, the control of gamete quality is of great importance (Migaud et al., 2013). Availability of good quality male and female gametes is necessary to close the lifecycle of a species and obtain subsequent generations. Variation in the availability or quality of gametes can jeopardize subsequent production and limit the possibilities for performing genetic selection. Given the high cost of broodstock rearing, large variations in the quantity or quality of gametes can significantly impact the competitiveness and sustainability of fish farms and aquaculture companies (Bromage et al., 1992). As a consequence, controlling the "on demand" production of good quality gametes is a major issue in aquaculture with important economic consequences (Migaud et al., 2013). While availability and quality of both male and female gametes are important, this is especially true for the female gamete. The female gamete has indeed several features that make its production and availability more difficult to control and optimize than the male gamete. While the male gamete, sperm, can be frozen in most, if not all, fish species, it is currently impossible to freeze female gametes, eggs, due to abundant yolk reserves present in the cytoplasm and high water content in the case of pelagic/floating eggs. In addition, eggs contain maternal factors and other compounds of maternal origin that vary in abundance based on environmental factors and broodstock management techniques.

The present article will focus on the female gamete with special attention paid to issues related to the control of its quality including (i) how to measure egg quality, (ii) how egg quality is influenced by environmental factors and aquaculture practices, (iii) the challenges for the future of aquaculture, and (iv) new areas of investigation and expected outputs.


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## Diversity of Fish Species and Variety of Egg Characteristics

More than 30,000 ray-finned fish (Actinopterygii) species have been reported, thus accounting for half of vertebrate animals on earth. A vast majority of ray-finned fish are teleosts with only 50 non-teleost species reported. Ray-finned fish evolution has spanned more than 400 million years (Near et al., 2012). Ray-finned fish are one of the most successful radiations of vertebrates, and a wide diversity of aquatic habitats and fish reproductive strategies are found in the wild (Jalabert, 2005). Fish diversity is also associated with tremendous differences in fish egg characteristics and features (Table 1), as well as in the duration of egg formation that can range from several weeks to several years (Lubzens et al., 2010), depending on the species. In addition, ray-finned fish exhibit a wide variety of reproductive strategies and behaviors. While some fish are able to produce eggs several times during their reproductive season, others can spawn only once per year, or once in their life. Differences in reproductive strategies can sometimes be observed in closely related species. For instance, within the *Oncorhynchus* genus, many semelparous species exist (e.g., the Pacific salmons) that can spawn only once and then die. In contrast, the rainbow trout (*Oncorhynchus mykiss*) is iteroparous and can reproduce annually.

## A Phenotype Difficult to Measure

As indicated above, egg quality can be defined as the ability of the egg to be fertilized and subsequently develop into a normal embryo. Under aquaculture conditions, poor egg quality can lead to several types of problems

## Table 1. Diversity of fish egg characteristics.*

| Name | Species | Fecondity (eggs/kg) | Egg diameter (mm) | Spawning | Egg features |
|---|---|---|---|---|---|
| Eel | *Anguilla anguilla* | 2,650,000 | 2 | Sea water | Floating and pelagic |
| Turbot | *S. maximus* | 1,000,000 | 1 | Sea water | Floating and pelagic |
| Cod | *Gadus morhua* | 500,000 | 1,5 | Sea water | Floating and pelagic |
| Seabass | *D. labrax* | 200,000 | 1,1-1,3 | Sea water | Floating and pelagic |
| Carp | *Cyprinus carpio* | 175,000 | 1,35 | Freshwater | Adhesive |
| Perch | *Perca fluviatilis* | 145,000 | 1,5-2 | Freshwater | Egg ribbons |
| Zebrafish | *Danio rerio* | 120,000 | 0.8-1 | Freshwater | Demersal |
| Trout | *O. mykiss* | 3,000 | 4-5 | Freshwater | Demersal |
| Tilapias | *Oreochromis sp* | 50-200 | 2-4 | Freshwater | Mouth breeding |

*Modified from (Jalabert, 2005).

such as lack of fertilization, problems of egg activation, development arrests, embryonic mortalities, and embryonic deformities, as previously reviewed (Kjörsvik et al., 1990; Brooks et al., 1997; Bobe and Labbé, 2010; Migaud et al., 2013). Given the wide variety of possible problems observed, extensive characterization of embryonic development is needed to fully assess egg quality, as some environmental factor and husbandry practices can induce specific malformations (Bonnet et al., 2007a). Similarly, specific mutations can induce very discrete malformations or developmental problems that could be difficult to detect by a macroscopic observation (Golling et al., 2002). In addition, differences in the dynamics of embryonic mortality cannot be detected when survival is assessed at a single developmental stage. Furthermore, there can be a delay between the occurrence of a developmental problem and the time when it can macroscopically be detected (Kleppe et al., 2013). This is especially true for problems occurring at late developmental stages. Evaluation of egg developmental potential should thus be performed under optimal conditions to avoid bias due to detrimental incubation or larval rearing conditions such as water quality, temperature, or rearing systems (Figure 1). Finally, one important parameter is the individual variations observed between egg batches and within an egg batch. As just an example, the fertilization rate of brown trout eggs kept in the body cavity for 15 d after ovulation ranged between 10 and 95% (Escaffre and Billard, 1979). Within an egg batch, a survival rate of 50% means that only half of the embryos will develop successfully. In this context, it is extremely difficult to sample homogeneous eggs to study the mechanisms involved. For this reason, many investigators have compared good vs. bad quality eggs (Carnevali et al., 2001a, 2001b; Aegerter et al., 2005; Mommens et al., 2014). It is, however, possible to analyze the correlation between egg quality and gene expression levels (Bonnet et al., 2007b; Mommens et al., 2010). These variations further complicate egg quality assessment and analysis of the molecular mechanisms underlying egg quality. It should also be stressed that a full assessment of egg quality is time consuming and that possibilities of performing automatic phenotyping of embryonic development are currently extremely limited. For these reasons, egg quality is a parameter that is currently not included in most selection schemes.

Eggs released from the ovary at ovulation include maternal factors such as maternal mRNA, hormones, and yolk proteins that will be later utilized to support embryonic development (Brooks et al., 1997). The time lag between the moment when eggs are spawned and the moment when the different maternal components present in the eggs are used further increases the difficulty of estimating/measuring what is a good (or a bad) quality egg. Even more difficult is the accurate estimation of the contribution of maternal factors (i.e., linked to egg quality) to early developmental success. All animal embryos pass through a stage during which developmental control is handed from maternally provided gene products to those synthesized from the zygotic genome (Tadros and Lipshitz, 2009). In fish, this maternal-to-zygotic transition (**MZT**) occurs during the mid-blastula stage (Kane and Kimmel, 1993; Tadros and Lipshitz, 2009). Maternal factors are required for egg activation and fertilization and for the earliest stages of embryonic development (Lindeman and Pelegri, 2010), for zygotic genome activation (Lee et al., 2013; Bouleau et al., 2014), and for developmental success beyond zygotic genome activation (**ZGA**; Wagner et al., 2004). When problems occur in development after ZGA, it is difficult to evaluate the respective contributions of maternal factors and zygotic genome expression to developmental success. It is also difficult to evaluate the respective contributions of male and female gametes. For this reason, investigations of egg quality often include non-limiting sperm quantities, pools of sperm originating from different males, and sperm with good motility (assessed before fertilization) to limit male-induced biases. Therefore, even though paternal contribution could be underestimated, it should be stressed that in contrast to female gamete, the male gamete mostly contributes to egg quality through its DNA and thus acts through genetic or epigenetic mechanisms. Sperm from various animals, from worms to humans, also contains several RNA species. The functional contribution of these factors to developmental success remains, however, controversial (Lalancette et al., 2008).

## The Influence of Environmental Factors and Aquaculture Practices

Egg quality is deeply influenced by environmental factors, husbandry practices, and the domestication level of the species (reviewed by Bobe and Labbé, 2010; Migaud et al., 2013). The effect of each environmental factor will not be reviewed here, but the importance of temperature, photoperiod, and diet will, however, be highlighted. Fish are poikilotherms; thus, water temperature directly influences the dynamics of the reproductive cycle and age at puberty (in relation to growth). In some species, the occurrence of specific phases of the reproductive cycle within a specific range of temperatures, or below a threshold temperature, are required to cue the reproductive cycle or to achieve optimal egg quality and subsequent juvenile production (Brown et al., 2006; Migaud et al., 2013). In addition, some critical steps of the cycle exist (e.g., final oocyte maturation) that can be very sensitive to temperature (Gillet, 1991). Exposure of female broodfish to suboptimal water temperatures, even for a short time, can lead to major problems in egg quality. For example, exposure of female rainbow trout to 17°C (i.e., in the range of the temperature of the species) for a few weeks before ovulation leads to reduced embryonic survival, increased malformation rate, and increased occurrence of triploid fry (Aegerter and Jalabert, 2004). The reproductive cycle is also very sensitive to photoperiodic cues. In many species, photoperiod is the main factor triggering sexual maturation (i.e., development of the gonad, ultimately leading to gamete production) alone or in combination with temperature (Bromage et al., 2001). Photoperiod can, therefore, be manipulated to obtain out-of-season breeding or egg production. While useful, such techniques can trigger egg quality problems, even though the importance of such phenomena can vary depending on the species, the type of artificial photothermal regime, and the physiological status of broodstock when treatment is applied (Bromage et al., 1992; Bobe and Labbé, 2010; Migaud et al., 2013).

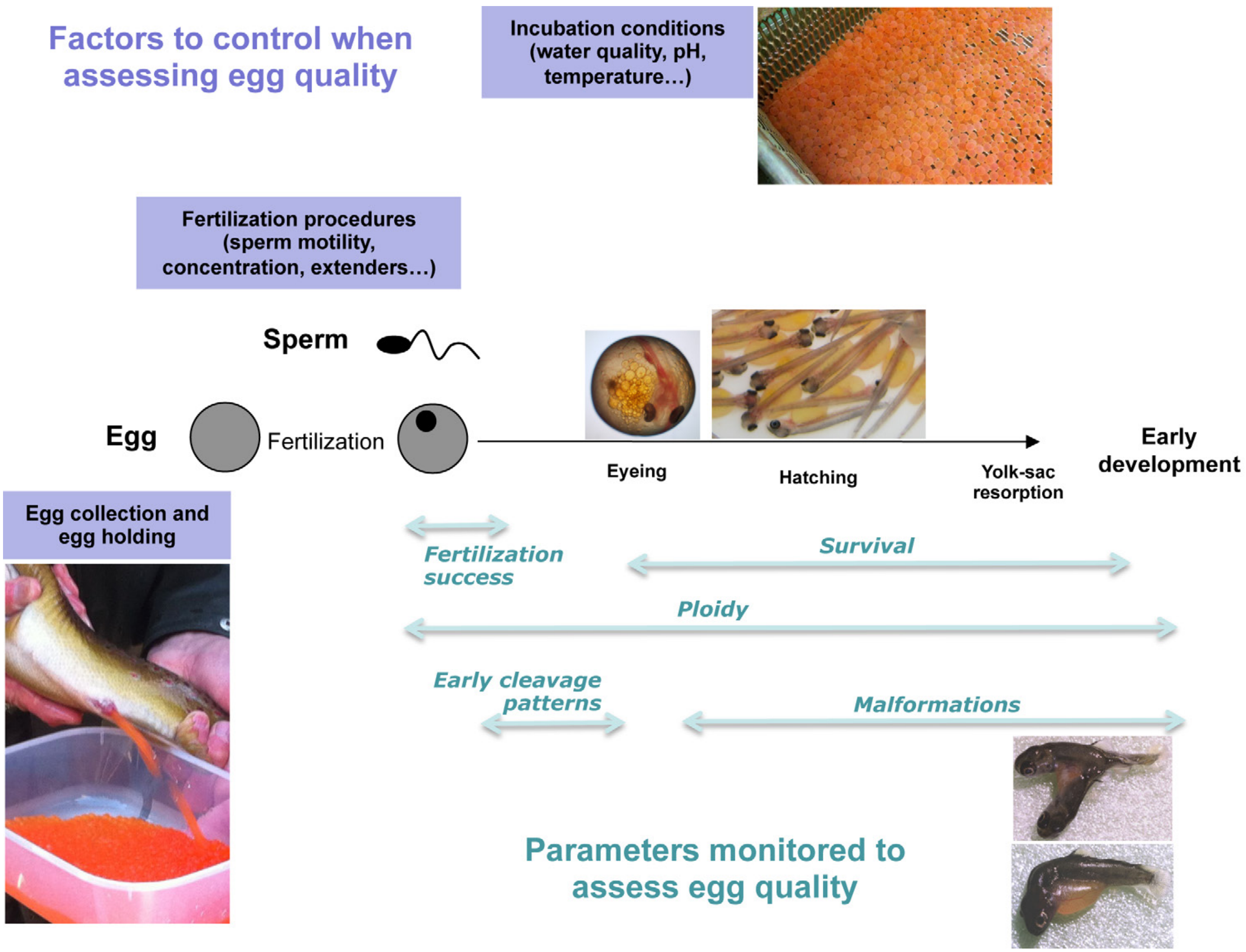



**Figure 1.** Overview of the factors that have to be controlled and parameters that are monitored when assessing egg quality.

Fish nutrition can directly influence egg yolk composition and therefore impact egg quality because embryos will subsequently utilize egg yolk until first feeding. It is therefore important that broodstock diets are optimized to ensure good larval survival and early development (Izquierdo et al., 2001). Thus, broodstock diets should be formulated to ensure all essential nutrient requirements are met for the species being cultured (Migaud et al., 2013). The lack of key components (e.g., vitamins) in the diet or suboptimal feeding levels can lead to major reproductive problems (Izquierdo et al., 2001). This is especially true for new aquaculture species in which the nutritive needs of the broodstock are unknown (i.e., in the case of newly domesticated species) or cannot be satisfied under aquaculture conditions. When appropriate food type and quantities are supplied to broodfish, problems of egg quality are, however, much more limited. Very few examples exist in which broodstock diet specifically triggers egg quality problems without inducing any reproductive problems earlier in the reproductive cycle (Brooks et al., 1997). Broodstock management techniques in general can induce egg quality problems. The overall impact on egg quality is highly dependent on the species, the techniques used, and the physiological status of the fish, including stress level. Interestingly, it was shown that environmental factors and husbandry practices resulted in specific egg quality problems such as specific malformations (Bonnet et al., 2007a). In addition, environmental factors and husbandry practices can influence the abundance of specific maternal mRNA (i.e., messenger RNA found in oocytes and early embryos that is derived from the maternal genome during oogenesis) in the egg (Bonnet et al., 2007b). Indeed, a relationship exists between gene expression profiles in the ovary and the quality of the egg produced at the completion of the reproductive cycle (Chapman et al., 2014). Similarly, differences exist in egg composition between populations exhibiting different domestication levels that could be linked to differential egg quality (Crespel et al., 2008). Together, these data indicate that the effects of environmental factors on egg quality are mediated, at least in part, by the maternal factors accumulated in the egg during its formation.

## A Challenge for the Future of Aquaculture

As indicated above, egg quality is deeply influenced not only by environmental factors and broodstock management techniques, but also by the aquaculture system used and the domestication level of the species or the population. It should also be stressed that each of these factors may act in a species-dependent manner. In the context of global climate change, species diversification and new production systems being developed, the ability to control egg quality remains a major issue in aquaculture (Migaud et al., 2013).

There is an increasing demand to expand aquaculture practices to include additional species (e.g., tuna), and the production of some "recent" aquaculture species (e.g., panga) has grown rapidly within a very short period of time. For "historical" aquaculture species, the control of egg quality is a possible way of improving the sustainability of the system. This is especially true for species (e.g., salmon) in which the female spawns once per year and has to be raised for several months or years to produce eggs, thus inducing major economic losses when final egg quality is poor. Even more problematic is the absence of egg quality parameters in genetic selection schemes, which can lead to fertility problems as previously observed in other animal production systems (Weigel, 2006). In addition, genetic studies that have analyzed the heritability of reproductive traits in aquaculture fish are scarce, especially for reproductive traits linked to developmental success (Kanis et al., 1976; Gall and Gross, 1978; Su et al., 1997). While most breeding programs have been shifting toward the development of genomic selection programs, this is especially problematic in the context of ray-finned fish aquaculture, given the increasingly high number and diversity of aquaculture species and the relatively low number of genome sequences available to date (see below).

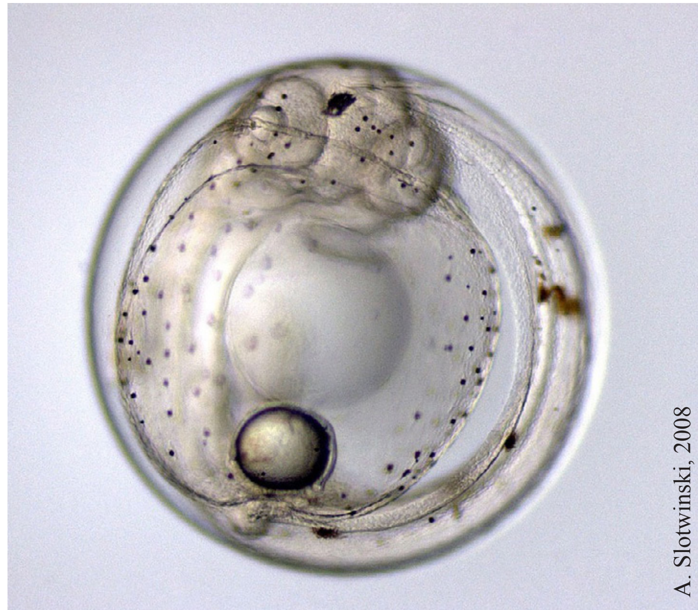
In the context of global climate change, many issues are raised that can impact egg quality. This is especially true for aquaculture systems in which the broodstock is directly exposed to the natural environment, such as seawater temperature for fish raised in cages. Global warming can lead not only to permanent changes in water temperature, but it can also result in a more variable temperature during the reproductive cycle. As discussed above, exposure to temperature outside an acceptable range, even for short period of time, can induce major egg quality problems that could require the development of new aquaculture systems or the selection of less sensitive (i.e., more robust) animals. Already problems with climate variation and water availability are driving the development of new aquaculture systems (e.g., recirculating aquaculture system) that can possibly raise novel issues in terms of egg quality control. We are, therefore, facing increasing challenges related to the control of egg quality in aquaculture, but several opportunities exist that will be discussed below.

## A Scientific Challenge with New Opportunities for Non-model Species

As previously stressed, many species are used in aquaculture and this figure will probably increase in the future due to high consumer demand and decreasing capture of wild fish (Chevassus-au-Louis and Lazard, 2009). Given the high number of ray-finned fish species (more than 30,000) and their high ecological success, the number of candidate species for aquaculture is theoretically very high. This raises many questions related to the scientific strategies that should be developed to cope with egg quality issues that will be further discussed below.

### *The advent of high-throughput sequencing will make the genome of most aquaculture species available*

Fish are widely used to address scientific questions in a variety of scientific fields such a vertebrate developmental biology, environmental biology and ecotoxicology, and even human diseases. As a consequence, the genome sequences of several fish species such as the zebrafish (*Danio rerio*) have been made available. However, the number of available fish genome sequences available in general, and of aquaculture species in particular, remains extremely limited. To date, the genome sequences of only a few economically relevant fish species have been published (Star et al., 2011; Henkel et al., 2012; Berthelot et al., 2014; Brawand et al., 2014). The recent development of next-generation sequencing techniques and the concomitant dramatic drop of sequencing costs now offer new possibilities to improve this situation. In the (very) near future, it will be possible to generate a draft genome sequence at a reasonable cost and using a simple strategy that can be handled by a local sequencing facility rather than by an international consortium involving major national sequencing centers. This will offer new possibilities in a field where a wide diversity of biological models exist and in which all efforts cannot be allocated to a single or a limited number of species. Availability of genome sequences for most economically relevant species is a prerequisite for the development of genomic selection in the future and the possible incorporation of complex traits, such as egg quality, in selection programs.




Fish produce many eggs, typically about 1mm across, and usually release them into the open water.

### *New possibilities of investigations*

Investigations designed to gain new insight on what makes a good (or a bad) quality egg are usually based on a differential analysis applied to molecular, biochemical, transcriptomic, or proteomic data. Such analyses, when fruitful, can demonstrate correlations between egg quality (i.e., developmental potential) and specific egg features such as the abundance or presence of one (or several) maternal factor(s) in the egg. To further demonstrate the importance of a specific maternal factor (i.e., gene product), or to describe the phenotype associated with this factor, it is subsequently relevant to block or inhibit its action. Depending on the type of maternal factor involved, several techniques can be used. For a maternally inherited mRNA, it is possible to inhibit its translation into protein (i.e., knock-down, **KD**) through the injection of antisense morpholino oligonucleotide (**MO**) into the one-cell embryo. This strategy was used to demonstrate the critical role of *nucleoplasmin*


(*npm2*) maternal mRNA for early developmental success in zebrafish (Bouleau et al., 2014). This study was initiated after the earlier report of a differential abundance of *npm2* maternal mRNA in rainbow trout eggs of low and high quality (Aegerter et al., 2005). It is, however, not always possible to use a KD strategy. This is especially true when the target maternal factor is acting at the protein level (i.e., when the abundance of the protein in the egg is correlated with developmental success) and is already present in the egg at fertilization. In this case, expression of the maternal factor has to be inhibited during oogenesis. While other gene knock-out strategies exist, the recent development of the CRISPR/Cas9 technology is very promising due to reasonable costs and proven high efficiency of the technique (Hwang et al., 2013). The CRISPR/Cas9 technology could offer great opportunities to characterize the contribution of specific maternal factors to egg developmental competence, even in fish species of economic importance. This technology was recently successfully used in commercially important species. In tilapia, this technology was used to investigate the role of several genes controlling germ line formation (Li et al., 2014), and several genes involved in pigmentation were targeted in Atlantic salmon (*Salmo salar*; Edvardsen et al., 2014). Together, this suggests that in addition to being very efficient in model species with a short generation time, this technology is also suitable to large aquaculture species with long generation times.

## *Fish egg quality in the light of evolution: Aiming for generic mechanisms or for species-specific features?*

Given the (very) high number of candidate aquaculture fish species, it is probably not relevant or even feasible to thoroughly investigate the mechanisms defining egg quality in each individual species. One of the challenges that we are facing is to identify and study generic mechanisms shared by evolutionarily distant species and for which deep investigation in one species could lead to insights applicable to a large number of species. For example, *npm2* is a maternal effect gene (i.e., a maternal gene of major importance for embryonic success) originally identified in the mouse (Burns et al., 2003). The link between *npm2* mRNA abundance and developmental success in the rainbow trout egg (Aegerter et al., 2005) and the maternal role of *npm2* demonstrated in zebrafish using a KD approach (Bouleau et al., 2014) suggests that the important role in egg developmental competence played by this maternal factor would be evolutionarily conserved and thus shared by many fish species. Looking at the mechanisms defining egg quality in the light of evolution is therefore one of the challenges that we are facing. While difficult, such an approach will greatly benefit from the genome sequences that will soon be made available in a wide variety of fish species, thus allowing investigators to take advantage of the specific features of one species to investigate mechanisms shared by a group of species. To be truly efficient, the quest for generic mechanisms must rely on well-defined orthology relationships among the genes present in analyzed species. The use of common reproductive traits shared by different species could also be a useful tool to investigate generic mechanisms shared by several species (Teletchea et al., 2008).

In contrast, some important factors leading to problems of egg quality could be specific to a single species or even a subpopulation. For example, the problems in egg quality observed when final oocyte maturation occurs at temperatures above 8°C in Arctic charr (*Salvelinus alpinus*) (Gillet, 1991) are not observed in brook trout (*Salvelinus fontinalis*), a closely related species. Similarly, each candidate aquaculture species might have specific requirements in terms of diet composition or might be more or less adapted than closely related species to specific aquaculture systems. Such situations will require investigations conducted in a species-dependent manner.

## *Automatic phenotyping*

As indicated above, a thorough analysis of embryonic development, including malformation, is necessary to fully characterize egg developmental competence. This is time consuming and therefore cannot be routinely performed in the field. One possibility is to develop automatic phenotyping systems that take advantage of image analysis technologies (and possibly wireless data transfer) to determine the occurrence of embryonic death and/or malformation. Such systems would require important methodological developments but would also allow, when available, the incorporation on egg quality parameters into selection programs.

### *Early predictors of developmental success*

Estimating egg quality through the monitoring of developmental success is time consuming and technically difficult. To overcome this issue, one possibility is to use predictive markers of egg quality (i.e., egg features that would be predictive of its developmental potential). Many investigators have tried to link morphological parameters of the egg to its developmental potential as previously reviewed (Bobe and Labbé, 2010). In most cases, if not all, morphological parameters can be used to identify eggs of extremely bad quality (i.e., eggs undergoing atresia) but are not relevant to discriminate eggs exhibiting a range of different developmental potentials (Ciereszko et al., 2009). In addition, the morphology of the first embryonic cells (i.e., the blastomeres) can easily be monitored in fish species with transparent embryos (Shields et al., 1997; Kjørsvik et al., 2003; Rideout et al., 2004), but normal cleavage does not necessarily imply a good developmental success (Avery et al., 2009).

Other parameters such as the pH in the fluid surrounding the eggs are easy to monitor and are correlated to negative factors impacting egg quality (i.e., post-ovulatory ageing of the egg) but are not always correlated with good egg developmental competence (Fauvel et al., 1993; Aegerter and Jalabert, 2004).

Several studies have led to the identification of differentially expressed mRNA (Aegerter et al., 2005; Bonnet et al., 2007b; Mommens et al., 2010) or proteins (Castets et al., 2012) in eggs of low and high quality. Differences in enzymatic activities have also been identified among eggs of varying quality (Carnevali et al., 2001a, 2001b). These analyses have been very useful to shed light on the molecular mechanisms defining egg developmental competence (i.e., egg quality). However, several issues exist that limit the use of these differential profiles as molecular markers of egg developmental competence. First, it is clear that different factors able to impact egg quality can act through different molecular mechanisms and therefore induce the differential expression of different mRNA or protein. To overcome this issue, it would be necessary to develop a composite index that incorporates molecular markers reflective of different quality problems. Second, it will probably be necessary to use highly discriminative markers (i.e., exhibiting a marked differential abundance in the egg) and to use a combination of markers to accurately predict egg quality. For example, expression profiling of a large set of genes in the striped bass ovary was recently used successfully to predict the quality of eggs produced at the end of the reproductive cycle (Chapman et al., 2014). Finally, development of such markers or sets of markers requires high standardization and high generic value (i.e., validity in a wide range of situations). The complexity of the techniques used to monitor gene and protein expression is a major bottleneck for the use of molecular markers in the field. This is a major challenge, but the rapid development of sequencing techniques could lead to important methodological advances in the near future. To date, these new techniques are not used for practice at the farmer's level. Missing steps toward application include not only the validation of robust markers, but also the development of user-friendly tools and equipment to be used under hatchery conditions.

## Conclusions

Broodstock management and gamete quality are major issues in aquaculture and important bottlenecks in the reproductive cycle of new aquaculture species. This is especially true for the female gamete in the current context of a changing environment and the simultaneous need of new aquaculture systems and demand for new species. The multifactorial determinism of egg quality further limits our knowledge of the mechanisms defining egg developmental competence. Understanding what makes a good quality egg remains a challenge, but opportunities exist that should be taken to pinpoint the generic mechanisms defining the developmental potential of the egg and ultimately develop robust molecular markers of egg quality in fish.

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Julien Bobe obtained his M.Sc. degree in biology at the University of Rennes 1 in France in 1997. Between 1998 and 2000, he conducted a research project on the molecular mechanisms of ovulation and post-ovulatory ovarian physiology under the supervision of Professor F.W. Goetz at the University of Notre Dame (IN, USA). After obtaining his Ph.D. from the University of Rennes 1 in 2001, he joined INRA (Institut National de la Recherche Agronomique), where he still works, as a permanent researcher to develop a research program on the molecular mechanisms underlying oocyte quality in fish. In 2010, at age 36, he became research director and took the lead of a research group working on sex differentiation and oogenesis in fish. His current foci are maternal-effect genes and evolution of gene function after whole-genome duplication in teleost fish. Dr. Bobe has published more than 55 papers in international journals, several book chapters, and has mentored more than 20 Ph.D. and M.Sc. students.

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