Aquaculture 428–429 (2014) 88–96

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Early prediction of long-term family growth performance based on cellular processes — A tool to expedite the establishment of superior foundation broodstock in breeding programs

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ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 17 February 2014
Accepted 20 February 2014
Available online 7 March 2014

Keywords:
Progeny testing
Genetic correlation
RNA:DNA
Cell cycle
Larvae
Selection

ABSTRACT

In the establishment of selection programs where foundation broodstock are taken from the wild, or from unevaluated stocks, there is no prior way to ascertain their genetic merit for growth rate. However, for highly fecund multiple spawning species, selection of broodstock founders could be made more efficient if their estimated breeding values (EBVs) could be determined through progeny testing early in the production cycle. Early progeny testing could allow farmers working with unimproved species to immediately re-spawn best EBV ranked broodstock for stocking grow-out systems, thereby avoiding costs associated with rearing slow-growing families. In this study, we quantified the additive genetic (co)variation of barramundi, *Lates calcarifer*, larval traits which could reveal their parents innate genetic capacity for fast growth. Specifically, we investigated the heritability (h^2) of cellular, biochemical and morphological larval traits (total RNA, total DNA, total protein, RNA/DNA, protein/DNA, the proportion of cells dividing and standard length, *Ls*) and their genetic correlations (r_g) with two morphological traits at harvest indicative of long-term growth (*Ls* and wet weight, *W*). Here, two cohorts originating from partial factorial crosses between 11 dams and 26 sires were sampled at 18 days post hatch (dph) in the hatchery then later at 273–469 dph at harvest. Pedigrees were reconstructed through microsatellite based parentage analyses and genetic parameters estimated through animal models via REML. All larval traits were heritable at 18 dph ($0.19 < h^2 < 0.51$), indicating that their expression is under additive genetic control and therefore that they could have predictive power to estimate parental EBV. This was confirmed by positive and significant r_g for all larval traits (except protein/DNA) and that of fish harvest size ($r_g > 0.60$, $P < 0.01$). In particular, high r_g were found between larval cellular and biochemical traits RNA/DNA, total RNA and the proportion of cells dividing ($0.81 < r_g < 0.88$, $P < 0.001$), indicating that larval families with higher metabolic rates also grew to be the larger and heavier families at harvest. Results showed that genetic differences in growth traits among barramundi broodstock could be determined shortly after spawning by measuring larval indicator traits predictive of long-term genetically determined growth. These larval predictive traits may allow fish breeders working with highly fecund multiple spawners like barramundi to explore the advantages of early progeny testing to expedite the establishment of superior foundation broodstock in breeding programs.

1. Introduction

Productivity of fish farming is intrinsically linked to survival and growth performance. Fast growing strains developed by aquaculture breeding programs utilize farming input resources like feeds more efficiently and overall significantly reduce production time and costs (Gjedrem et al., 2012). However, the majority of world aquaculture production is based on unimproved stocks, which exhibit high levels of variability in growth and survival among batches. In the establishment of foundation broodstock for hatchery populations or breeding nucleus of selection programs, there is currently no prior way to ascertain the animal's genetic merit for growth rate. Classically, broodstock are spawned and progeny reared for a long time, often to harvest sizes, before individuals are measured and estimates of breeding values (EBV) of the animal and families can be determined as a basis for selection. Permanent and continuous genetic gains are only realized after a few selected generations, usually in the order of a decade. Thus, the development of genetically superior farmed stocks is still a long way away for most aquaculture industries, especially those working with species with long generation intervals (e.g. 2–4 years).

To date, breeding programs in aquaculture have not yet exploited the enormous potential of progeny testing for the selection of genetically superior broodstock. In established terrestrial livestock, however, the

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http://dx.doi.org/10.1016/j.aquaculture.2014.02.037
0044-8486/

evaluation and selection of breeders through the performance of their offspring is the driver of several genetic improvement programs (Norman et al., 2001). For example, progeny testing is the primary source of genetic gain within the Australian dairy industry (ADHIS, 2012). In fact, for traits with moderate heritability (h^2), as is the case for growth-related traits (e.g. harvest weight, length) in aquatic species (Gjedrem and Olesen, 2005), progeny records are considered the ultimate source of information for the calculation of an animal's EBV (Bourdon, 2000). This is because progeny testing is the most accurate method to estimate the breeding value of an animal (e.g. more accurate than individual, within-family, sib-selection or combined selection approaches), provided records from a number of progeny from half-sib families are taken (Gjerde, 1991, 2005). Nevertheless, large scale family-based breeding programs, first developed for salmonids (Gjedrem, 2010) and now established as the industry standard (Gjedrem, 2012), generally spawn each breeder only once, missing out on the opportunity to generate and promptly disseminate consecutive cohorts of fast growing fish from those highly (EBV) ranked breeders to grow-out farms. For fishes like salmonids this approach is used because high mortality rates are expected after spawning, which in most cases impedes the generation of a second progeny cohort from the same breeders (Gjerde, 2005). For a large number of highly fecund multiple spawning species which reproduce for longer periods (e.g. months to years) there is, however, obvious potential for progeny testing. For aquaculture industries working with such species, breeding programs using foundation stock from progeny tested broodstock can theoretically yield faster rates of genetic gain than contemporary breeding programs (Macbeth and Palmer, 2011). Therefore, the use of progeny tested breeders could allow for a more immediate realization of increases in aquaculture productivity and profitability.

One shortcoming of progeny testing in aquaculture has been attributed to longer generation intervals, possibly doubled that of traditional schemes, because selection of broodstock is not performed until offspring have been measured (Fjalestad, 2005; Gjedrem, 1983). This is particularly true if progeny are tested after individual growth trajectories have been confidently established (e.g. one or two year old progeny) and progeny are sexually mature. However, this principle does not apply if progeny testing was performed very early in the production cycle and the best (EBV) ranked broodstock immediately re-spawned to produce the cohorts to be stocked by farmers. This could be achieved by exploring potential genetic correlations between economically important traits (e.g. fish harvest weight) and traits measured at larval stages. When an inherited trait expressed early in life is influenced by the same set of genes which also affect a trait later in life, such early and late traits are genetically correlated, that is, there is correlation between breeding values for those traits, even when there may be no observable phenotypic links between them (Falconer and Mackay, 1996). Therefore, if a larval trait is heritable and highly genetically correlated with a harvest growth trait, the progeny larval records could be used to determine parental EBVs, allowing for pre-screening and identification of broodstock with the best growth characteristics. This method of selection could enable the improvement of fish growth rates as soon as the subsequent spawning cycle and potentially lead to immediate increases in productivity. For biologically suited species, early progeny testing could prove an important additional tool for traditional breeding programs and expedite the establishment of superior foundation broodstock.

Increasing evidence suggests that fish larval traits are under the influence of a strong genetic component which could reveal their parents' innate genetic capacity (EBV) for fast growth. Numerous studies have shown that family origin and/or paternal (i.e. purely genetic) effects play a significant role in fish growth as early as embryonic and larval stages (Butts and Litvak, 2007; Eilertsen et al., 2009; Green and McCormick, 2005; Núñez et al., 2011; Ottesen and Babiak, 2007; Probst et al., 2006; Saillant et al., 2001; Trippel et al., 2005) and reported that heritabilities of diverse traits measured very early in life (e.g. Ma et al., 2008; Páez et al., 2010; Shimada et al., 2007; Table S1) share similar moderate magnitudes as those reported for adults (Gjedrem and Olesen, 2005). Most importantly, positive genetic correlations between adult breeder size and offspring early life history traits (e.g. larval size, weight gain, swimming performance and survival) have been demonstrated in both captive (Munch et al., 2005; Vandeputte et al., 2002) and wild (Johnson et al., 2010, 2011) fish populations, although genetic links between different ontogenetic stages among marine organisms still remain largely unknown (Marshall and Morgan, 2011).

In fisheries sciences, larval size is undoubtedly the simplest and most studied of all early life history traits (Miller et al., 1988). Larval size, however, may not necessarily be the most predictive trait to disclose long-term determined genetic growth. In fact there is a range of more informative and measurable cellular and biochemical larval traits underlying biological mechanisms driving an organism's increase in body size and which ultimately lead to the expression of observable morphometric characters, such as length and weight. Larval fish increase their body mass through increases in protein biosynthesis (Caldarone, 2005; Houlihan et al., 1988), with the level of protein synthesis largely determined by the availability and transcriptional regulation of RNA (Elser et al., 2000; Henshaw et al., 1971). Unlike that of its precursor template DNA, which remains relatively stable in an organism over time, RNA levels fluctuate dramatically depending on the metabolic activity and growth rate of cells (McNamara et al., 1999), especially during early life stages when mass-specific metabolism is at its highest (Johnston, 2006). In addition, post-embryonic growth in teleosts is largely manifested through cell division (hyperplasia) and growth of muscle tissue, where myoblasts from a proliferating population of myogenic progenitor cells fuse with muscle fibers as fiber diameter and length increase (Johnston, 2006). Therefore, faster growing larvae are expected to exhibit higher RNA:DNA ratios (RNA/DNA) and higher rates of cell division than slow growing larvae.

The development of sensitive fluorometric and cytometric techniques has allowed fish biologists to measure the rate of such cellular metabolic processes early on in development. In particular, RNA/DNA and protein:DNA ratio (Prot/DNA) have been extensively validated as indirect metabolic indices indicative of nutritional condition and growth potential for a broad range of fish species (reviewed by Chícharo and Chícharo, 2008; Ferron and Leggett, 1994; Perez-Dominguez and Dahm, 2011). More recently, flow cytometric (FCM) cell cycle analyses have also proved to be a powerful tool to directly assess growth of fish larvae by quantifying the relative proportion of cells which are actively dividing, i.e. cells found in the S (DNA synthesis phase) and G_2–M (gap 2 and mitosis) replicative phases of the cell cycle (Bromhead et al., 2000; Domingos et al., 2012; González-Quirós et al., 2007; Porter and Bailey, 2011; Theilacker and Shen, 2001). To date, all of these studies have focused on the phenotypic correlations of larval growth and their corresponding cellular and metabolic responses to environmental conditions (e.g. food availability, temperature, etc.), without addressing the potential for a correlated genetic response with long-term growth performance. Based on significant parental effects (Høie et al., 1999) and moderate heritability (Bang et al., 2006) found for Atlantic herring (*Clupea harengus*) larval RNA/DNA at hatch ($h^2 = 0.31$), study authors have suggested that the genetically determined expression of growth-related processes early in life could also persist in later life stages, affecting overall fish growth trajectories (Bang et al., 2006; Høie et al., 1999). Although this hypothesis has never been tested in fish, selection experiments for higher RNA/DNA content in mammary glands of female mice over 13 generations in mice resulted in significant increases in mammary gland weight and body weight of mothers at second parturition (Sung, 1970). Genetic differences between fish individuals leading to disparities in growth rate are well known (Gjedrem, 1997). If the kinetics of these processes is enacted from early development it is conceivable that they could predict the long-term genetic growth potential of progeny and/or their parents. Therefore, fish that are increasing body mass due to