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Heritability of harvest growth traits and genotype–environment interactions in barramundi, *Lates calcarifer* (Bloch)

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ABSTRACT

Due to its popularity as a food fish, rapid growth and wide environmental tolerances, barramundi, *Lates calcarifer*, production levels are increasing worldwide and breeding programs are currently being considered and implemented throughout Asia and Australia. However, for selective breeding programs to be effective, it is essential to have information on genetic parameters such as heritability and genetic correlations of traits, as well as on how families perform relative to each other over the culture cycle in single or in multiple environments (ti). Genetic parameters and G × E interactions for barramundi traits (namely weight (W), standard length (Ls), body depth (BD), Fulton's condition factor ($K = 10^6 W / Ls^3$) and a body shape index ($H = 10$ BD / Ls)) at 62 days post hatch (dph) and at harvest size (273–469 dph) were estimated for the first time for this species based on microsatellite DNA parentage assignment of 3110 offspring generated in three mass spawning events, where up to 121 families were produced per batch. Heritability estimates for growth related traits W, Ls and BD were moderate for fish reared in cages at 62 dph (average $h^2 = 0.22, 0.27, 0.15$; respectively) and high at harvest for fish reared in intensive tanks and in a semi intensive pond (average $h^2 = 0.40, 0.37, 0.40$; respectively). Estimates for ratio traits K and H were lower than for growth traits for all ages and environments (average h^2 K = 0.14 and h^2 H = 0.09). Genetic and phenotypic correlations between W and Ls, W and BD and Ls and BD ranged from 0.91 to 0.99, whereas correlations involving K and H and other traits (W, Ls and BD) were lower (0.07 to 0.88), but positive, indicating that these traits may also be modified if selection is based on W (or Ls) alone. In addition, no significant G × E interactions for growth related traits W, Ls and BD were detected for barramundi either reared in fresh *vs.* sea water cages at 62 dph ($r_g \geq 0.97$), or commercially reared in fresh water until harvest size (343–469 dph) in an intensive recirculation aquaculture system *vs.* a semi-intensive pond ($r_g \sim 0.99$). High heritability estimates found here show that additive genetic effects play a significant role in barramundi growth, especially in older fish, suggesting that growth rates could be greatly improved through selective breeding.

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1. Introduction

Barramundi, *Lates calcarifer*, is a highly valued and popular aquaculture species farmed throughout the Indo-West Pacific region, with production rapidly increasing worldwide. Barramundi is a catadromous species, having a high tolerance to culture in either fresh or salt water farming conditions that can vary from pond, tank or cage-based culture systems. In addition, its high fecundity (females spawn an average of 300,000 eggs per kg of body weight), fast growth rates (reaching up to 2 kg on farm in 12 months) and good market acceptance have led to the development of a growing aquaculture industry (Garcia, 1990; Grey, 1987; Rimmer and Russell, 1998; Schipp et al., 2007). Barramundi aquaculture commenced in Thailand during the 1970s and rapidly spread throughout Southeast Asia and Australia, although global production (65,857 tonnes in 2010) remains primarily based on unimproved farmed stocks (FAO, 2012). However, as has been shown for several aquaculture species, there is enormous potential for selective breeding programs to improve commercially important traits, such as growth rate (Gjedrem and Thodesen, 2005). By growing fish that have been genetically improved with selective breeding over several generations, it has been possible to increase the efficiency of production per unit farm area and per total input resources (feed, labour, etc.), thus greatly improving the economic value of fish farming (Gjedrem et al., 2012). Nevertheless, many barramundi farmers still rely on fingerlings produced from wild caught broodstock which exhibit high levels of variability in performance among batches, both within and between hatcheries, followed by uncertainty of long-term growth and survival.

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Barramundi is a protandrous hermaphrodite which reproduces by mass spawning in nature. The lack of complete control over sexual maturation, differentiation and reproduction presents challenges for the selective breeding of barramundi (Robinson and Jerry, 2009; Robinson et al., 2010). Strip spawning is difficult to perform for barramundi, and despite a single report on successful artificial fertilisation trials using cryopreserved sperm (Palmer et al., 1993), current breeding practices in Australia still rely on mass spawning using one or two females and four or more males in ~20,000 l tanks (Macbeth et al., 2002). This practice results in the production of several maternal and paternal full- and half-sib families and minimises non-genetic early environmental effects which could arise if individual families were kept separately until animals were large enough to be tagged for later communal rearing. However, the practice of mass spawning in a breeding nucleus has been discouraged (Gjerde, 2005) because the relative contribution from each brooder to the total number of offspring is unknown; therefore if individual selection is to be performed, selected fish for the next generation may be the offspring of a limited number of parents (Gjerde, 2005) reducing effective population sizes and increasing the risk of inbreeding and its associated undesired consequences (Gjedrem and Baranski, 2009; Sonesson et al., 2005; Wang et al., 2002). In practice, studies on several aquaculture species, including barramundi, have overcome this problem through genotyping of broodstock and offspring with microsatellite DNA markers and retrospectively constructing pedigree relationships with parentage assignment software (Frost et al., 2006; Jerry et al., 2006a; Kvingedal et al., 2010; Lind et al., 2010; Wang et al., 2008).

Despite these challenges, efforts to improve commercially important farmed traits in barramundi through selective breeding are currently underway in Asia (Yue et al., 2009) and close to implementation in Australia (Australian Seafood Cooperative Research Centre and Australian Barramundi Farmers Association). Quantifying the amount of genetic variation determining the phenotypic expression of commercially important traits (e.g. heritability and genetic correlations) is fundamental for the design of barramundi selective breeding programs (Robinson and Jerry, 2009). To date, the impact of different rearing environments on the realisation of genetic potential in barramundi has not been investigated. Such knowledge, however, is of particular importance as barramundi is farmed under a broad range of culture systems. If G × E interactions are considerable, breeding programs will need to be tailored to account for each of the different environments in which the animals are to be commercially produced (Gjedrem and Olesen, 2005).

Heritability estimates for weight, length and condition factor for juvenile barramundi (~18 g and 10 cm length) at 90 days post hatch (dph) have been estimated for Asian barramundi stocks by Wang et al. (2008). Estimation of genetic parameters for early growth traits is important because selecting future breeding candidates at an early age would decrease fish maintenance costs in a breeding nucleus. In addition, size grading and culling of smaller fish (i.e. a form of early mass selection) are common procedures in commercial barramundi culture to avoid cannibalism and production losses and is usually first undertaken around the third week post hatch (Schipp et al., 2007). Early size grading significantly impacts genetic diversity of barramundi (Frost et al., 2006), therefore these current culture practices (i.e. grading and culling) are also likely to impact on barramundi genetic parameters by the end of the culture cycle. To date, little is known about how additive genetic variability in barramundi affects economically important traits at harvest size (i.e. closer to sexual maturity, when breeding candidates are generally selected) and how families perform relative to each other over the culture cycle in similar or disparate environments. Therefore, in this study we estimated heritability of barramundi traits at 62 dph and at harvest (273–469 dph) and genetic correlations for body weight, standard length, body depth, Fulton's condition factor and body shape index at harvest. Furthermore, we looked for evidence of G × E interactions in barramundi reared in fresh water *vs.* sea water cages and in a semi-intensive pond *vs.* intensive tank system.

2. Material and methods

2.1. Mass spawning of broodstock and larval rearing

Offspring fish in the present study originated from three mass spawning events which took place in two commercial hatcheries in Queensland, Australia. In both facilities, broodfish were first anaesthetised with 40 ppm of AQUI-S® (AQUI-S) for assessment of spawning condition (see below) and fin clipped for later DNA parentage analyses. All fin tissues collected throughout this study were preserved into individually labelled microfuge tubes containing DMSO-salt solution (20% DMSO, 0.25 M disodium-EDTA and NaCl to saturation at pH 8) (Seutin et al., 1991) until DNA was extracted. To ascertain spawning condition, females were cannulated using a plastic tube (1.2 mm internal diameter) and oocytes examined under a light microscope. Females bearing loose, spherical and greater than 400 μm oocytes were induced with a single intramuscular injection of luteinizing hormone release hormone analogue (LHRHa) at a dosage of 50 μg/kg. More than 50% of males freely expressed milt by gentle pressure on the abdomen and no males were hormonally induced. Spawning occurred in the broodstock holding tanks around dusk during the second and third nights after injection. Buoyant eggs from mixed families (i.e. with the least possible degree of environmental bias among families) were collected by skimming the water surface, eggs that sunk were discarded. Fertilisation rates for the first nights spawn were generally low (<50%) and only the eggs from the second night (fertilisation rates > 85%) were kept for all three batches. Larval rearing followed intensive clear water marine finfish larviculture protocols, which included periodic grading after 18 dph to avoid cannibalism (Dhert et al., 1992; Schipp et al., 2007), until 26 dph when offspring were sent to different rearing environments in three separate experiments (approximate fish size range at 26 dph: 6–18 mm standard length and 0.05–0.20 g wet weight). The number of dams and sires present in tanks at spawning, the offspring rearing environment and type of analysis performed in each experiment are shown in Table 1.

2.2. Experimental groups, grow out environments and fish sampling

2.2.1. Experiment 1 — genetic parameters at harvest size

Offspring for experiment 1 (spawn 1: Table 1) were reared in tanks at an intensive (50–100 kg fish/m^3) indoor recirculation grow out facility in Melbourne (VIC, Australia, Farm 1) and harvested at 273 dph. At Farm 1 fish were maintained in fresh water with oxygen concentrations > 5 mg/l and temperature 28 ± 2 °C and fed at least twice daily. Fish were periodically sorted into large, medium and small size categories (grading), allowing fish to be recombined after each grading event. Smaller size classes were occasionally discarded (i.e. culling of slow growers) until fish reached an average weight of 200 g. Grading was performed mechanically and the criterion was

Table 1
Barramundi *L. calcarifer* offspring rearing conditions, number of dams and sires present in the spawning tank and type of analysis performed in each experiment. Tank = commercial intensive tank culture, Pond = semi-intensive pond culture, Cage = cages within 2000-L tanks in controlled environment, FW = fresh water, SW = sea water, h^2 = heritability, r_g = genetic correlation between traits, r_p = phenotypic correlation between traits, G × E = genotype by environment interactions.

Experiment/spawn	1	2	3
Rearing systems	Tank	Tank *vs.* Pond	Cage FW *vs.* SW
Number of dams	12	6	10
Number of sires	21	6	13
Analysis	$h^2/r_g/r_p$	h^2/G × E	h^2/G × E

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