

Next-generation transcriptome profiling reveals insights into genetic factors contributing to growth differences and temperature adaptation in Australian populations of barramundi (*Lates calcarifer*)

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ABSTRACT

Identification of genetically-regulated adaptation in fish is a precursor to understanding how populations will respond to future climate induced stressors like temperature. Australian populations of barramundi (*Lates calcarifer*) show strong evidence of local adaptation to temperature. However, the phenotypic consequences of this adaptation are unknown and the genetic mechanisms underlying this response are poorly understood. In the current study, two populations of barramundi from temperature extremes of the species Australian distribution were communally reared at cool (22 °C), control (28 °C) and hot (36 °C) water temperatures for 3.5 months. Southern populations of barramundi originating from a cooler environment grew significantly faster at 22 °C than northern populations of warm adapted barramundi. However, no difference in population growth was present at either 28 °C or 36 °C. The underlying transcriptome profile of barramundi was examined via Illumina mRNA deep sequencing to determine the major contributing gene categories giving rise to phenotypic differences in barramundi population growth. Gene ontology (GO) analysis revealed enrichment in categories relating to the regulation of peptidase activity as well as microtubule, cytoplasmic and cellular metabolic based processes. Further analysis of the GO category “microtubule based process” with associated genes from the “response to stress” category revealed an apparent re-organization of cytoskeletal elements in response to an induced cold stress in northern barramundi reared at 22 °C, when compared with northern barramundi reared at 36 °C. Between southern barramundi and northern barramundi reared at 36 °C, an analysis of the “endopeptidase inhibitor activity” GO category in conjunction with stress genes indicated a suppression of the complement system in southern barramundi along with an increase in the cellular stress response. The results of the present study show that southern populations of barramundi exhibit underlying molecular adaptation to cooler water temperatures, but still retain a tolerance for warm water temperatures. Furthermore, GO profiling has revealed groups of genes that underlie population differences in temperature tolerance as a means to prioritize the analysis of differential gene expression in studies of local adaptation in the future.

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1. Introduction

The effects of temperature on poikilothermic organisms are felt at every level of biological organization, from animal behavior and physiology to the cellular expression of genes and proteins (Huey & Bennet, 1990). For tropical estuarine species such as barramundi (*Lates calcarifer*), coping with fluctuations in environmental temperature is paramount to their survival as estuarine water temperatures vary significantly on a daily and seasonal basis.

Climate change is expected to further exacerbate these already frequent variations in environmental conditions, and is thus likely to pose a significant challenge for local barramundi populations in the near future (Bianchi, 2006). Australian populations of barramundi (*L. calcarifer*) range from the Ashburton River (22° 30' S) in Western Australia, across the tropical north of the country, and down the eastern Queensland coast to the Noosa River (26° 30' S). Throughout this distribution barramundi inhabit fresh, estuarine and near coastal waters over some 16° of latitude that encompass a wide range of environmental temperatures. At the northern and southern end of their Australian distribution, mean yearly average temperatures differ significantly and range from 23.2–32 °C in Darwin, Northern Territory, to 18.5–27.7 °C in Gladstone, central Queensland, respectively (Bureau of Meteorology, <http://www.bom.gov.au>). As a species, barramundi experience significantly warmer and more consistent temperatures at lower latitudes

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while encountering cooler and less consistent temperatures at higher latitudes. Across this thermal cline barramundi has also been shown to exhibit significant genetic structuring, with up to 16 discrete genetic stocks identified to date (Keenan, 1994; Salini and Shaklee, 1988) (Fig. 1). In addition to this, barramundi are euryhaline and catadromous species and require estuarine and in-shore marine habitats to breed. However, after eggs hatch, juvenile barramundi migrate upstream to freshwater river systems away from river mouths (Pusey et al., 2004) and on the basis of recorded tagged fish movements it is believed that the migration of individuals between adjacent river-mouths more than 100 km apart, while possible, is a relatively rare event (Keenan, 1994). Therefore, gene flow amongst adjacent populations appears to be restricted, leading to the patterns of genetic structure exhibited in this species. Taken together, these observations have prompted speculation as to whether the high levels of genetic structure within populations of barramundi have translated into functional genetic adaptation to local environmental stressors, for example temperature.

Examination of the current barramundi stock structure in Australia through biogeographical studies suggests that phenotypic differences arising between populations from genetic differences should be relatively small. This is due in part to the relatively recent establishment of the current population structure (arising ~17,000 years ago) and evidence for substantial migration and hybridization between historical eastern and western populations during the last glacial maxima (Keenan, 2000). However, studies by Rogers and Bloomfield (1993) and later Newton et al. (2010), show that populations from different thermal environments respond differently under thermal stress for traits such as survival, growth and upper thermal tolerance. Rogers and Bloomfield (1993) reared two Queensland strains of barramundi (from Cairns, northern Queensland and Burrum River, central Queensland — see Fig. 1) in open freshwater cage culture while recording environmental conditions and the phenotypic performance of both fish populations. Over the entire culture period both populations exhibited similar growth rates, however, bacterial infections caused greater mortality during cold weather periods in the

northern Cairns strain. As temperatures cooled with the onset of winter, Burrum River fish were observed to have higher feed rates, while Cairns fish had lower appetite, lower condition factor, reduced growth during winter and higher mortality rates. The authors suggested that their findings were indicative of the unique adaptation of Cairns and Burrum River strains to local thermal conditions (Rogers and Bloomfield, 1993). Newton et al. (2010) using thermal challenge experiments showed that the upper thermal tolerance of barramundi populations from the extreme latitudinal ranges of the species Australian distribution significantly differed. Barramundi from lower latitudes (warmer conditions) exhibited greater tolerance to high water temperatures than fish from higher latitudes (colder conditions). These results lend strong support to the argument that Australian barramundi do in fact show evidence of local adaptation to temperature.

The relationship between local environment and thermal tolerance in fish has also been revealed in a few other species. In common killifish (*Fundulus heteroclitus*) critical thermal maxima and minima were shown to be different between northern and southern populations over a range of acclimation temperatures. The underlying genetics revealed differences in *Ldh-B* concentration (Crawford and Powers, 1992) and heat shock protein (*Hsps*) expression between populations, showing that killifish thermal tolerance limits have a substantial genetic basis and vary in a direction consistent with what is predicted for fish that have undergone localized adaptation to environment (Fangue et al., 2006). A genetic analysis looking at the effects of acclimation to various cold water temperatures in carp (*Cyprinus carpio*) found a large body of genes underlying this response. Specifically, in muscle many genes were found to be involved in the remodeling of the contractile apparatus, hence improving physiological performance at low temperatures. With such a large volume of genetic information generated, gene ontology (GO) categories were constructed revealing that the acclimation of carp to cold temperatures had significant contributions from genes involved in transcription regulation, RNA splicing and translation (Gracey et al., 2004). Furthermore, adjustments in the

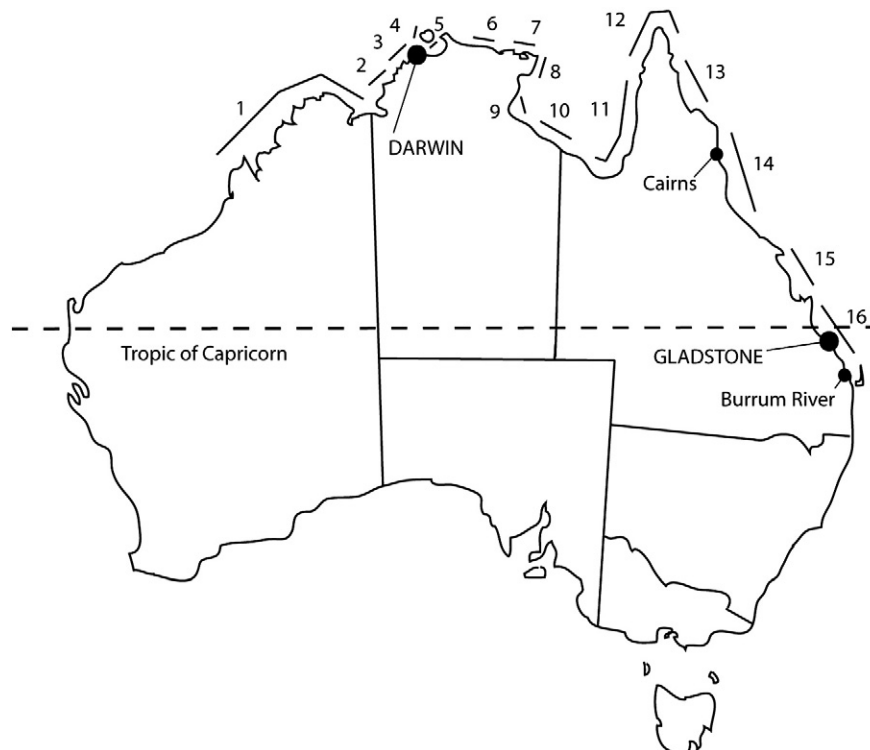


Fig 1. Taken from Newton et al. (2010). Australian populations of barramundi based upon Keenan (2000). 1, Fitzroy to Ord and Moyle Rivers; 2, Daly and Finniss Rivers; 3, Darwin Harbour and Shoal Bay; 4, Port Hurd; 5, Mary River, NT; 6, Goyder River; 7, Buckingham Bay; 8, Blue Mud Bay; 9, Roper River; 10, McArthur River; 11, south-east Gulf of Carpentaria; 12, Cape York Tip; 13, east Cape York and Princes Charlotte Bay; 14, north east coast; 15, central east coast; 16, south east coast. The locations of Darwin, Gladstone, Cairns and Burrum River are also shown.

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