



Heat tolerance and gene expression responses to heat stress in threespine sticklebacks from ecologically divergent environments



Karin Brydsø Dammark, Anne-Laure Ferchaud¹, Michael M. Hansen, Jesper G. Sørensen*

Department of Bioscience, Aarhus University, Ny Munkegade 114, DK-8000 Aarhus C, Denmark

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ABSTRACT

Ectothermic animals like fishes are extremely dependent on temperature, as they are not able to change body temperature physiologically. When populations are found in isolated water bodies such as small lakes they will have to respond to stressful high temperatures by behavioral avoidance, phenotypic plasticity or microevolutionary change. We analyzed threespine sticklebacks from two large and two small lakes, representing different isolated populations. We determined maximum critical thermal limits (CTmax) and the associated gene expression responses in three heat shock (*hsp60*, *hsp70*, *hsp90*) and two key metabolic (*idh2*, *fbp2*) genes at ecologically relevant moderate heat stress (26 °C) as well as at the critical thermal limit (CTmax). CTmax showed slight variation across populations with no strong indication of local adaptation. Likewise, there was no strong evidence for local adaptation at the level of gene expression. The expression of the metabolic genes indicated a shift from aerobic towards anaerobic energy production with extreme heat stress. We conclude that threespine sticklebacks do not show severe stress during the warmest temperatures they are likely to encounter during current temperature regimes in Denmark, and following this show no sign of local adaptation even in small, isolated water bodies.

1. Introduction

Anthropogenic climate change is increasingly causing elevated global temperatures (Kerr, 2007; Marcott et al., 2013; Moss et al., 2010), and effects on plants and animals are already evident (Geerts et al., 2015; Parmesan, 2006; Sydeman et al., 2015). The effects of climate change on living organisms are complex and manifold. Temperature in itself, and particularly extreme summer temperatures may cause heat stress (Deutsch et al., 2008; Hoffmann and Sgro, 2011; Pörtner and Knust, 2007; Pörtner and Peck, 2010). Whether organisms will cope with rapid changes in environmental conditions via short-term plastic responses (i.e. acclimatization), local adaptation, or a combination of these mechanisms is not well understood. In addition, it is not clear if local adaptation can occur rapidly enough to track changes in climate. Some studies have demonstrated genetic change at ecologically important traits and markers coinciding with climate change (Balanya et al., 2006; Bradshaw and Holzapfel, 2001; Geerts et al., 2015; Pulido and Berthold, 2010; Umina et al., 2005), but in many other cases it is unclear if observed responses reflect phenotypic plasticity or evolutionary genetic change (Crozier and Hutchings, 2014; Hansen et al., 2012; Merila and Hendry, 2014).

Fishes are [with a few exceptions (Dickson and Graham, 2004)] ectothermic and thereby unable to change body temperature physiologically. The physiology of fishes is therefore highly influenced by temperature that affects general metabolism and thereby activity, reproduction and growth (Ficke et al., 2007; Hill et al., 2008; Wootton, 1998). So far, most research on temperature adaptation and climate change adaptability in fishes has focused on relatively few model systems. These studies have found local or environment-dependent temperature-related adaptation in life-history traits (Cote et al., 2016; Haugen and Vøllestad, 2000; Jensen et al., 2008; Koskinen et al., 2002; Metzger et al., 2016a). Examples include associations between thermal regimes and loci under possible diversifying selection (Bradbury et al., 2010; Hecht et al., 2015; Narum et al., 2010), responses to selection for important temperature-related physiological traits (Munoz et al., 2015), differences in gene expression reaction norms between populations (Fangue et al., 2006; Meier et al., 2014; Niu et al., 2008; Papakostas et al., 2014), and associations between thermal regimes and oxygen limitation (Anttila et al., 2013; McBryan et al., 2016; Pörtner and Knust, 2007; Schulte, 2015). Altogether, these results demonstrate potentials for plastic and evolutionary responses to climate change, although only recently studies have specifically focused on how populations and

* Corresponding author.

E-mail address: jesper.soerensen@bios.au.dk (J.G. Sørensen).

¹ Present address: Institut de Biologie Intégrative et des Systèmes (IBIS), Pavillon Charles-Eugène-Marchand, 1030, Avenue de la Médecine, Local 1145 Université Laval, Québec (Québec), Canada G1V 0A6.

species respond when extreme summer temperatures occur (Brown et al., 2016; Fanguie et al., 2011; Gallant et al., 2017; Tomalty et al., 2015).

Here we investigate a temperate model fish species, the threespine stickleback, *Gasterosteus aculeatus*. This species has a wide geographical distribution, shows pronounced ecological, phenotypic and behavioral variation (Bell and Foster, 1994; Östlund-Nilsson et al., 2007) and its genome has been sequenced and assembled (Jones et al., 2012). Furthermore, its generation time is short, generally 1–3 years in temperate regions (Bell and Foster, 1994; DeFaveri and Merila, 2013) which makes it a candidate for adapting to local environmental conditions and environmental change on a contemporary time scale. In Denmark it is found in a wide range of habitats, including coastal marine regions, rivers, larger freshwater lakes and small lakes and ponds that are often man-made and isolated with no possibilities for migration. In particular, small isolated ponds may experience high temperatures during particularly warm periods of summer. We therefore investigated the heat stress response in threespine stickleback populations from four isolated environments: two small, shallow and two larger, deeper lakes. Experiments were conducted with wild-caught individuals, which were acclimated to a common environment prior to the experiments in order to reduce environmentally induced differences.

From a physiological perspective, maximum critical thermal limit (CT_{max}) is assumed to be a key parameter when assessing the maximum temperature a population can survive in the short term during e.g. extreme climatic events. However, empirical support for adaptive variation in heat tolerance of sticklebacks is lacking. Earlier studies reported evidence for cold tolerance adaptation but not heat tolerance adaptation in the form of CT_{max} in threespine stickleback (Barrett et al., 2011; Gibbons et al., 2016). Metzger et al. (2016a) found differences in CT_{max} as induced by acclimation to different salinities, but no differences in CT_{max} between marine and freshwater sticklebacks. Hence, CT_{max} might be similar across different populations, but if differences should exist, we expect to find them among populations genetically adapted to different thermal environments. In the present study, we expected that fish from smaller lakes experienced higher temperatures during summer, and we hypothesized that sticklebacks from environments experiencing the warmest temperatures would also be the most resilient to heat stress, either through previous acclimatization and/or through genetically based adaptation. Second, we investigated the response to high temperatures at the level of gene expression. The investigated genes encompassed three Heat Shock Proteins (HSPs). HSPs are an important part of the cellular stress response and responsible for the maintenance of homeostasis within the cell and up-regulated proportionally to accumulation of stress (e.g. heat) induced damage (Sørensen et al., 2003). Further, we investigated the gene expression of two metabolic proteins (fructose biphosphatase-2 and isocitrate dehydrogenase) involved in key energy production pathways (glycolysis/gluconeogenesis and the citric acid cycle, respectively). Previous studies confirm that the expression levels of different heat shock paralogs and metabolic genes generally are sensitive to temperature in fish (e.g. Tomalty et al., 2015). To address the acute response we investigated gene expression at the temperature where fish reached their critical thermal limit (CT_{max}; defined as observed loss of equilibrium). Measuring responses to sub-lethal temperatures could be more useful than CT_{max} when studying implications of elevated temperature for the long-term survival of populations. Thus, we also investigated the responses in gene expression to more short-term chronic exposures (24 and 48 h) to a temperature within the upper range that sticklebacks would encounter in their native environments (26 °C).

We hypothesized that all populations would up-regulate HSPs under maximal stressful conditions. For the metabolic genes, we expected expression to be up-regulated with increasing sub-lethal temperature (short-term chronic exposure). With severely stressful conditions (CT_{max}) we expected expression of metabolic genes to be negatively correlated with up-regulation of HSPs, thus indicating a metabolic cost



Fig. 1. Map showing the location of the studied populations in Denmark: Lake Hald (HAL; geographical coordinates 56.367302, 9.333352), Lake Knud (KNU; 56.099821, 9.783578), Botanical Garden Lake (BOT; 56.160444, 10.190847) and Thors Mølle Lake (THO; 56.121454, 10.213345).

of the stress response. For the 24 and 48 h exposures to sub-lethal elevated temperature we expected population differences to reflect adaptive differences in acclimation ability. Normal levels of gene expression would indicate the ability to maintain metabolic activity, while up-regulation of HSPs and down-regulation of metabolic genes at a sub-lethal temperature could indicate sub-lethal stress.

2. Materials and methods

2.1. Study area and lake temperatures

Four collection sites were selected, all situated in Jutland, Denmark (Fig. 1). These included two small, shallow lakes situated in parks in the municipality of Aarhus: The Botanical Garden Lake (BOT) and Thors Mølle Lake (THO) (both ca. 900 m², max depth < 2 m). The lakes are fed by water from small inlets and in the case of THO possibly also groundwater. Migration into and from both lakes is restricted due to impassable dams. The remaining two collection sites were larger lakes and both parts of the Gudenaa River system: Lake Hald (HAL; 3.3 km², max depth: 31 m) and Lake Knud (KNU; 2.0 km², max depth: 29 m). HAL is isolated due to an impassable dam built ca. 500 years ago, and KNU is also isolated by dams ca. 5 km upstream (ca. 500 years old) and 10 km downstream (ca. 150 years old) in the Gudenaa River system. However, sticklebacks have the option to migrate to cooler areas during periods of high temperature, either by moving into deeper water or by migrating into cooler inlet rivers. Water temperatures at the four collection sites were measured using temperature loggers (HOBO U22, Onset Computer Corporation, Bourne, MA, USA), situated ca. 3 m from the shore on the lake bottom and set to log every 30 min. In HAL, temperatures were measured from 31/7/2014–23/8/2014, in KNU from 31/7/2014–15/8/2014, in THO from 25/6/2014–8/9/2014 and in BOT temperature loggers were deployed from 20/6/2014–8/8/2014. More temperature loggers were deployed but could not be recovered. Loggers were presumably discovered and removed by persons bathing in HAL and KNU and persons walking in the park areas surrounding THO and

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