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Discovery of latitudinal gradient of triiodothyronine concentrations in ectotherms as revealed from a cyprinid fish, the common roach *Rutilus rutilus*

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ABSTRACT

Important life history traits in groups of ectotherms have been shown to vary along a latitudinal axis. Despite sustained interest to this phenomenon, the underlying physiological mechanisms of latitudinal adaptation remain poorly understood. Thyroid hormones (THs) are key regulators of metabolism, development, and growth, and are involved in shaping adult phenotypes in lower vertebrates, fishes and amphibians. We tested the hypothesis that concentrations of triiodothyronine (T_3), the most active form of THs, correlate with latitudinal gradient in ectotherms using a cyprinid fish, the common roach *Rutilus rutilus* as an example. Fish from seven locations between 46°45′ and 58°04′ N were studied for T_3 concentrations. Our results show a strong positive correlation between latitude and T_3 concentrations. There was a three-fold difference between the means of the extreme southern and northern samples. This is a first finding of latitudinal gradient of thyroid hormones in ectotherms. Photoperiodism and temperature were tested as main environmental factors influencing TH levels. In our results, the increase in T_3 concentrations along the south–north axis in ectotherms is, seemingly, associated with adaptation to differing thermal environments.

1. Introduction

The biology of ectothermal animals is significantly affected by latitude due to climatic changes. Ectotherms are distributed world-wide possessing complex adaptations necessary for surviving at different environmental conditions. Some species exhibit wide-spread distribution and significant plasticity of life history traits implying effective intraspecies adaptations to environmentally variable water bodies along a latitudinal axis (Bozinovic et al., 2011; Gaitán-Espitia et al., 2014). The lifetime patterns of growth, development, and reproduction of organisms are some of the many important traits affected by latitude (Yamahira et al., 2007; Chavarie et al., 2010; Thomas et al., 2012). Many phenotypic traits of ectotherms also have been reported to be influenced by latitude (Lindsey, 1988; Billerbeck et al., 1997; Stoks et al., 2014). Temperature is one of the most important environmental factors acting on ectotherms along a latitudinal axis (White et al., 2012; Sunday et al., 2014).

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Although many studies have explored the phenomena of thermal adaptations (see reviews in Tattersall et al., 2012; Sunday et al., 2014), the underlying physiological mechanisms of these adaptations still remain poorly understood.

Hormones are a link between the genome and the environment (Dufty et al., 2002; Reinecke, 2010; Lema, 2014). Among others, the thyroid hormones (THs) play important roles in regulation of many physiological traits, such as metabolism, development, growth, and reproduction in lower vertebrates, fishes, and amphibians (Shi, 1999; Power et al., 2001; Blanton and Specker, 2007). The THs are crucial for amphibian and fish metamorphosis as well as less drastic transformations during larval-to-juvenile transition (Inui and Miwa, 1985; Laudet, 2011; McMenamin and Parichy, 2013).

Thyroid hormones (THs), thyroxine (T_4) and the more active form triiodothyronine (T_3), are in part responsible for thermogenesis in mammals. In some studies, endotherms exhibited lower serum T_3 concentrations at southern localities whereas concentrations were higher in the same species at northern locations (Dunlap et al., 2008; Fair et al., 2011; Zheng et al., 2013). The correlation of TH concentrations in plasma with latitude in endotherms was considered in these studies as an adaptation to the different environmental temperatures. There is only one study implying the relationship between latitude and THs level in an ectotherm. The concentrations of T_3 and T_4 in the yolk of bonnethead shark *Sphyrna tiburo* (L) from a Tampa Bay population (located in the more northern region) were consistently higher than those in the yolk from a more southern population in Florida Bay (McComb et al., 2005).

The serum levels of THs are known to fluctuate seasonally in fish (see reviews in: Leatherland, 1982; Eales and Brown, 1993; Comeau et al., 2000). These variations are associated with seasonal climate changes, thermal regime of water bodies, photoperiodism, breeding cycle (Cyr and Eales, 1996; Pavlidis et al., 2000; Eales, 2006), and migration (Baggerman, 1962; Comeau et al., 2001; Kitano et al., 2012). Although THs are involved in regulation of many physiological processes, serum TH concentrations positively correlate mainly with temperature during the annual cycle (Bau and Parent, 2000; Abbas et al., 2012; Bolotovskiy and Levin, 2015).

Seasonal or cyclical changes in TH levels have been recorded for many species. However, unlike birds and mammals, surprisingly little is known about the geographic variation of these hormones in fishes (McComb et al., 2005; Ishikawa and Kitano, 2012). Thyroid hormones are involved in stimulation of Na^+/K^+-ATPase activity in fish (Madsen and Korsgaard, 1989), a component of the cold acclimation response (Schwarzbaum et al., 1992). Recent experiments with cold water acclimated zebrafish *Danio rerio* (Hamilton 1822) showed THs to maintain high levels of locomotor performance, muscle function, metabolic capacity, and heart rate in response to cold (Little et al., 2013; Little and Seebacher, 2014). In fact, THs regulate cold acclimation in ectotherms using the same pathways as during thermogenesis in endotherms exposed to cold (Little and Seebacher, 2014).

Based on examples of latitudinal correlation of TH production in endotherms, and recent discovery of TH-dependent cold water acclimation in ectotherms we hypothesize that serum triiodothyronine levels correlate with a latitudinal gradient in ectotherms.

The cyprinid fish, the common roach *Rutilus rutilus* (L), is a common and widely distributed species in temperate latitudes of Eurasia and therefore serves as a good case to study latitudinal variation of TH levels of ectotherms in the temperate zone. Indeed, the common roach was established to serve as a wild model species in endocrine disruption studies (Trubiroha et al., 2010; Söffker and Tyler, 2012; Gerbron et al., 2014) but still has not been examined for serum T_3 levels. Therefore, we have also investigated seasonal variation in serum T_3 concentrations of this species in order to understand the limits of natural variation and to identify which period is more appropriate for latitudinal sampling.

2. Material and methods

2.1. Animals and geographic data

Seasonal variation was studied using 133 adult individuals of the common roach from one population at a locality, the Upper Volga, Rybinsk Reservoir (58°04′ N 38°16′ E). Fish were sampled approximately every month during two years, March, 2011 to March, 2013; however, sampling was more frequent during pre-spawning and through the spawning period. Spawning was detected during 8–9 and 10–12 May in 2011 and 2012, respectively. Pre-spawning samples were collected 5 and 7 days before spawning in 2011 and 2012, respectively. Blood was collected from fish exuding gametes. Each sample comprised 5 to 6 specimens of each sex. Seven populations were sampled for the latitudinal study at seven locations between 46°45′ and 58°04′ N in the European region of Russia during a short period at the end of summer (23 August – 4 September 2012) (Table 1, Fig. 1). This period is one of the most appropriate for latitudinal sampling as it is temporally distinct from the spawning season when fluctuations of TH levels occur (see below). Geographic coordinates were obtained using a GPS receiver (Garmin, Oregon 450, USA). Water at one of the localities (No. 7) was brackish but all other localities were fresh-water. Water temperature was measured using thermometer, and duration of daylight was recorded using http://planetcalc.ru/300 at each sampling location. Fish were caught using gill nets or seine, and blood was obtained from fish immediately after capture. Only healthy, parasite-free individuals (as visually inspected) were used for analysis. All animals were measured, and the sex and stage of reproduction ascertained visually. Only adult specimens were used for analysis. A total of 61 specimens were processed for the study of latitudinal variation.

All fish were collected with permission of local authorities and experiments were approved by the Ethical Committee for Animal Experiments at the Institute of Biology of Inland Waters, Russian Academy of Sciences.