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Aquaculture 244 (2005) 331–340

Aquaculture

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Ontogeny of tolerance to hypoxia and oxygen consumption of larval and juvenile red sea bream, *Pagrus major*

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Received 14 October 2004; received in revised form 17 November 2004; accepted 18 November 2004

Abstract

Changes in tolerance to hypoxic stress and oxygen consumption were studied in the red sea bream, *Pagrus major*, from its early life stage until 42 days post-hatch. In the experiments, metamorphosis was observed mainly from days 15 to 30, and the morphological shift from larva to juvenile was completed at around 9.5 mm total length (TL). During the larval stage, lethally low dissolved oxygen (DO) levels and mass-specific metabolic rates increased with growth from 2.6 to 5 mm TL ($P<0.01$). Subsequently, the levels remained high and decreased until about 9.5 mm TL around the flexion stage and post flexion stage. Finally, beginning in the juvenile stage, lethal DO levels and mass-specific metabolic rates decreased as TL increased up to about 30 mm ($P<0.01$). The relationship between lethal DO levels and mass-specific metabolic rates was significantly linear ($r=0.59$, $p<0.001$, $n=207$) in fish larvae and juveniles. These results indicated that, around the stage of flexion and post flexion larvae in red sea bream, metabolic rates were highest during metamorphosis, and consequently hypoxia tolerance was lowest. It was presumed that the increasing metabolic rate during metamorphosis induced a decrease in the metabolic scope of activity and thereby induced the decrease of the tolerance to some environmental stressors in the background.

Keywords: Stress; Hypoxia; Oxygen consumption; Larvae; Development; Red sea bream

1. Introduction

In teleosts, the larval stage is a period of dramatic morphological, biochemical, and physiological changes. Metamorphosis is the focus of many such changes, and some of these metamorphic changes are controlled by hormones such as thyroid hormones, cortisol, and prolactin (Inui and Miwa, 1985; Hiroi et al., 1997). Some chemical components, such as nucleic acid, proximate composition, and enzyme activity of whole body, are also altered significantly during morphological changes in the larval through

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doi:10.1016/j.aquaculture.2004.11.019

juvenile stages (Ehrlich, 1974; Fukuda et al., 1986; Richard et al., 1991; Tanangonan et al., 1998; Gwak and Tanaka, 2002).

Adaptability to various environmental changes is also altered during larval development (Ishibashi et al., 2003, 2004a). In particular, the tolerance to hypo- and hyper-salinity in some kinds of fish changes around the period of metamorphosis (Hiroi et al., 1997; Shikano and Fujio, 1999; Chona et al., 2000; Ishibashi et al., 2003). The responses of larvae to osmotic gradients have been well documented (Chona et al., 2000). Chloride cells were found in early development (Ayson et al., 1994; Bone et al., 1995). As the larvae grow into juveniles, the gills complete their development, mainly by an osmoregulation site related to salinity tolerance; this site is thought to move from the skin to the gills (Hwang, 1987; Jurss and Bastrop, 1995). Although some of the many changes that occur during larval development are extremely complicated and controversial, other kinds of adaptability to environmental changes have not been fully examined.

Understanding the environmental physiology of fish is also of practical importance to industry, because cultivated fish are frequently exposed to stressors induced by fluctuations in such environmental factors as dissolved oxygen level, salinity, and water temperature (Ishibashi et al., 1992; Pihl et al., 1992; Ishibashi, 1994; Wu, 2002). In addition, disease can be a consequence of these stress factors (Chen et al., 2002; Yada and Nakanishi, 2002). For the last decade, many kinds of marine juvenile fish have been intensively cultured from eggs in fish nurseries. However, many sorts of bacterial and viral diseases have been observed in larvae and juveniles (Muroga et al., 1998), and some deformities that are thought to originate from feeding methods occur during nursery production (Matsuoka, 1987; Koumoundouros et al., 1997; Gavaia et al., 2002; Shimizu and Takeuchi, 2002; Haga et al., 2004).

To protect the health of nursery-produced fish, a lot of articles have appeared about many kinds of diseases and a few protective methods in the rearing of fish larvae and juveniles (Tatner, 1996; Muroga et al., 1998; Watanabe et al., 1998). The effects of enriched rotifer and *Artemia salina* lipids on activities of some marine fish from larvae through juvenile stages have also been examined (Izquierdo et al., 1989; Takeuchi et al., 1990; Tago et al., 1999). However, in the seed production of some marine cultivated fish, survival rates have not been stable enough, and further studies about larval health have been needed. Therefore, knowledge of stress tolerance and of the adaptation function in fish larvae through juvenile stages is extremely important for healthy nursery production.

We previously examined the ontogenic changes in various stress tolerances of larval and juvenile red sea bream, *Pagrus major*, and found that all tolerances to temperature, salinity, and ammonia stresses temporarily declined at metamorphosis (Ishibashi et al., 2003). It is considered that the depression of the scope for activity based on the increased metabolic rate during metamorphosis induced the decreases in various stress tolerances in the background. Previously, we also studied the effects of hypoxia on stress response and energy metabolism in young red sea bream (Ishibashi et al., 2002a,b) as well as in the Japanese parrot fish, *Oplegnathus fasciatus* (Ishibashi, 1994) and the Nile tilapia, *Oreochromis niloticus* (Ishibashi et al., 2002c). We found that in these fishes, exposure to hypoxia was associated with cortisol release, a reduction in oxygen consumption and liver ATP levels for metabolic depression, and increased anaerobic metabolism (Ishibashi et al., 2002a,b,c). However, there has appeared no study of ontogenetic tolerance to hypoxic stress in fish from the larval through juvenile stages.

In the present study, in order to obtain basic knowledge as to why some stress tolerances fall during metamorphosis, we examined ontogenic changes in tolerance to hypoxic stress and oxygen consumption among larval and juvenile red sea bream.

2. Materials and methods

2.1. Fish and feeding methods

Fertilized red sea bream eggs were obtained, reared, and studied at three facilities of Kinki University, Japan. First, eggs from the university's Fish Nursery Center were placed in several 500-l polycarbonate tanks (lot 1) at the Fisheries Laboratory, as well as in a 20,000-l concrete tank (lot 2) at the Center and in several 300-l polycarbonate tanks (lot 3) in the Fisheries Department's laboratory. In each case, gentle aeration was provided for 2 days until the eggs hatched. The day of hatching was regarded as day 0. In the pre-