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Interactive effects of incubation temperature and salinity on the early life stages of pacific cod *Gadus macrocephalus*

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ABSTRACT

The combined effects of incubation temperature and salinity on the early life stages of Pacific cod *Gadus macrocephalus* were examined under controlled laboratory conditions. Data were collected from two synchronized experiments. Experiment 1 was designed to evaluate the interactive effects of incubation temperature and salinity on the hatchability of fertilized *G. macrocephalus* eggs. Experiment 2 was set up to evaluate the interactive effects of incubation temperature and salinity on the time from hatching to 50% mortality of the non-fed yolk-sac larvae (M_{50}). The results show that temperature could significantly influence the development and hatchability of the larvae, as well as the hatching characteristics of *G. macrocephalus*. Viable hatch was significantly influenced by salinity when the upper and lower thermal limits were approached and shows the synergism of low salinity on egg development at low-temperatures and conversely inhibitory effects of low-salinity at high-temperatures. Data on developmental rates as influenced by temperature were presented at each tested salinity level. No influence of salinity was found at the temperature levels tested. Dome-shaped quadratic curves were fitted to the relationship between temperature and the incidence of larval size and yolk storage at hatch for most of the tested salinity levels. The effect of salinity across all temperatures, however, had a much smaller influence on larval size and no effect on yolk storage at hatch. The influence of temperature on larval duration (time from hatching to M_{50}) could be described in all cases by an exponential power function. Evidence on the synergism of low salinity at low-temperatures and conversely inhibitory effects of low-salinity at high-temperatures was also observed. The results were discussed in reference to salinity modified temperature effects on the early life stages of *G. macrocephalus*. Maximum hatchability and larval size at hatch, and moderate salinity tolerance and larval duration suggest an optimal temperature range of 4 °C to 6 °C for the survival and development of the early life stages of *G. macrocephalus* in the field.

1. Introduction

The eggs and larvae stages of the teleost life cycle are considered to be the most sensitive stages to environmental stressors during the teleost life cycle (Swanson, 1996). The Pacific cod *Gadus macrocephalus* Tilesius 1810 is a transoceanic species that can be found mainly along the continental shelf and upper slope of the North Pacific (Sakurai, 2007). *G. macrocephalus* is a total demersal spawner. The specific densities of eggs range from 1.0316 to 1.0454 g cm^{-3} (Bian et al., 2014a) and the eggs are partially adhesive until the blastoderm stage (Sakurai and Hattori, 1996; Bian et al., 2014a). *G. macrocephalus* generally migrate to relatively deep waters (80–290 m in depth) with silty or sandy bottom to spawn (Ketchen, 1961; Alderdice and Forrester, 1971; Klovach et al., 1995; Sakurai and Hattori, 1996; Laurel et al., 2010). An exception to this is in the southern part of their Asian range, where *G. macrocephalus* move inshore to spawn in shallow bays (15–50 m in depth) with silty or sandy bottoms (Alderdice and Forrester, 1971; Sakurai, 2007). The *G. macrocephalus* eggs would tend to remain on or near the sea sediment surface until hatching, after which the larvae would shift from a demersal to a pelagic existence and be found in the upper reaches of the water column

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(Laurel et al., 2010; Bian et al., 2014a). The larvae are epipelagic and transported to nearshore suitable nursery grounds by the current (Almatar, 1984; Abookire et al., 2007). Thus, the eggs and larvae may have greater potential to be exposed to varying water masses (Laurel et al., 2008), making them highly sensitive to spatial and temporal changes in their spawning habitat environment and exhibit high variation in survival at early life history stages (Laurel et al., 2008).

The response of different stocks to habitat environment variation differs in relation to their adaptations to the environmental conditions in their respective habitats (Rijnsdorp et al., 2009). A stock-specific habitat environment and optimal temperature range exist for survival and hatching in *G. macrocephalus* on both sides of the Pacific (Alderdice and Forrester, 1971; Sakurai, 2007; Laurel et al., 2008). Thus, the stock-specific responses to future patterns of environmental variability should be evaluated (Neer et al., 2007). From early December to mid-February, adult *G. macrocephalus* inhabiting the waters near northern Japan migrate and spawn in the mouth and inner parts of the Mutsu Bay with silty or sandy bottoms and water temperatures ranging from 4 °C to 8 °C (Takatsu et al., 2002). *G. macrocephalus* larvae are transported to the inner part of the Bay by the Tsugaru Warm Current (Takatsu et al., 2002). As one of several spawning grounds of this stock in Asia, Mutsu Bay (located near the southern boundary of the stock distribution) provides marginal benefits for *G. macrocephalus* in terms of temperature (Takatsu et al., 2002; Bian et al., 2014b). Sakurai (2007) reported that variations in *G. macrocephalus* catches of Japan, especially in Mutsu Bay, coincide with the timing of climatic regime shifts. These findings raised concerns as to how *G. macrocephalus* responds directly to the changing environment during its early developmental stages on the northern Japan stock.

Temperature is arguably the most important environmental influence that drives the development, growth, and survival of marine fish during their early life history (ELH) (Hemmer et al., 1990). Understanding the thermal niche of a species and how additional environmental factors reduce or enlarge that niche is a basic prerequisite for understanding the habitat requirements and how the productivity and distribution of species may be altered because of climate change (Kim et al., 2004). Temperature effects on fish eggs and larvae are modified by salinity; thus, the two factors are normally studied in combination (Pankhurst and Munday, 2011). Many laboratory studies have evaluated the effect of temperature on the survival and rates of ELH stages development of *G. macrocephalus* distributed in the northeast Pacific (Forrester, 1964; Forrester and Alderdice, 1966; Alderdice and Forrester, 1971; Laurel, et al. 2008; Hurst et al., 2010) and some also the combined effect of additional environmental factors (e.g., salinity and oxygen) alter the influences within these stocks (Forrester and Alderdice, 1966; Alderdice and Forrester, 1971). A common pitfall of the aforementioned experimental designs is that they did not include the full range of temperatures (including those that are lethal) experienced in the wild. Given that the ELH of Pacific cod remains largely unknown to the northern Japan stock, early studies were carried out to demonstrate the laboratory-validated data regarding the development, hatching, and survival responses of fertilized Mutsu Bay *G. macrocephalus* eggs (Bian et al., 2014b). However, these studies are insufficient to describe how Mutsu Bay *G. macrocephalus* will respond directly to the changing environment during its early developmental stages. Moreover, the optimal environment for the culture of Mutsu Bay embryos and yolk sac larvae remains unclear.

In this paper, comprehensive studies were designed to examine the effects of both temperature and salinity conditions on the timing of key events and survival of *G. macrocephalus* during their ELH. Separate experiments were conducted on fertilized eggs and newly hatched non-fed yolk-sac larvae of *G. macrocephalus*. The goal of this study was 2-fold: (1) determine combined effects of incubation temperature and salinity on the hatching success, length and yolk supply of the newly hatched larvae and how variations in temperature and salinities influenced survival and vital rates of *G. macrocephalus* during the eggs and early larval phase and (2) determine the combined effects of incubation temperature and salinity on larval duration (time from hatching to M_{50}) to find how the environment influences the survival of yolk sac larvae when they emergence to fluctuating sea surface and disperse into various water masses at the time of hatching. This study is necessary to gain a "cause-and-effect" understanding of the impacts of climate change on *G. macrocephalus* of the northern Japan stock. Moreover, these experiments will provide some basic knowledge for the culture of Mutsu Bay *G. macrocephalus* embryos and yolk sac larvae.

2. Material and methods

2.1. Egg collection

Adult *G. macrocephalus* were collected using bottom-set nets off the coast of Wakinosawa, Mutsu Bay, Japan (41°06′N, 140°49′E) during the spawning season from January to February 2009. The adults were transported to the shore and cultured in a large maintenance raceway tank (10 m long, 2.5 m wide, 1.2 m deep and 30,000 l in volume) at the Aquaculture Center of Aomori Prefecture. The fishes were transported from the aquaculture center to a 10,000-l circular tank at the Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University, using a 1000l tank carried by truck. The parental stock maintenance temperature was 6.0 °C and the salinity was 34 psu. The sex and maturity of the individual fishes were distinguished by inspecting germ cells sampled from the gonopore using a pipette. The body measurements of the total body length (L_T in cm), scale-covered body length (L_B in cm) and body mass (W in g) for the parental brood stock before and after the fertilization experiment were recorded, and the gonado–somatic index (I_G) was calculated as the ratio of gonad mass (W_G) to body mass (W), $I_G = 100\, W_G W^{-1}$.

The female and male broodstocks were all 5-year-old adults with L_T of 66.8–76.2 cm, L_B of 63.0–71.2 cm and an I_G of 12.07–18.59 (Hattori et al., 1992). Eggs of two female fish were collected in a dry plastic tray by manually stripping the abdomens of each mature female fish. The milt of three male fish was added over the eggs in the same manner. Fertilization was ensured by mixing the eggs, sperm, and filtered seawater. After approximately 10 min, the eggs were gently rinsed and tissue fragments were removed. The temperature and salinity of the seawater filtered using 0.22 μm microfiltration membranes were 6.0 °C and 34 psu, respectively, during the fertilization and incubation experiments.

2.2. Culture systems

Two experimental incubation systems were used in this study, namely static and flow-through. In the 'static' incubation system, temperature was maintained using the temperature-controlled incubator (*Sanyo* MIR154; www.sanyobiomedical.com). Embryos were cultured in polystyrene Petri dishes with temperature-adjusted seawater of experimental salinity. In the 'flow-through' incubation system, the fertilized eggs were placed in a hatching jar for incubation. A gentle upwelling flow of temperature-controlled seawater supplied to the jar kept the eggs moving and supplies oxygen. The weak upwelling circulation was maintained in the jar by positioning the in-flow at the bottom center of the jar with light aeration. The 'static' rearing system experiments were designed to study embryonic and early yolk-dependent larval