Journal of Applied Ichthyology

J. Appl. Ichthyol. (2014), 1–4
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ISSN 0175–8659

Received: July 14, 2013
Accepted: January 14, 2014
doi: 10.1111/jai.12488

Short communication

Gene pool and population structure in larval *Herklotsichthys quadrimaculatus* (Rüppell, 1837)

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Introduction

Small pelagic marine fishes comprise most of the world's landed fish catch (Freon et al., 2005), with members of the family Clupeidae among the dominant species (FAO, 2006–2013). The Clupeidae is most diverse in the Indo-West Pacific (Whitehead, 1985), a region proposed as being the geographic origin of this family (Lavoue et al., 2012). Earlier investigations revealed insignificant genetic differentiation, even across vast distances (Hedgecock et al., 1989). Such a lack of genetic differentiation is complementary to the morphological plasticity (Kinsey et al., 1994), suggesting that the Clupeidae is indeed capable of extended larval dispersal (Gonzales and Zardoya, 2007).

In the Philippines, members of the family Clupeidae in their larval stage are among the highest landed catches (Luna, 2009). Of these Clupeid larvae, the bluestripe herring *H. quadrimaculatus* (Rüppell, 1837) is the dominant species, especially abundant in the Verde Island Passages (Campos et al., 2011). This species has been reported to be highly adaptable and tolerant to a wide variety of environments (Milton et al., 1994). Dense concentrations of larval bluestripe herring on reef flats and mangroves have been observed in certain months (Williams and Clarke, 1983), and are reported to rely on a diverse array of food sources (De Troch et al. 1998). Such versatility allowed the successful introduction of *H. quadrimaculatus* beyond its native habitat (Williams and Clarke, 1983). However, no studies have been conducted to resolve the population structure and gene pool of this common herring.

The objective of this work has been to provide preliminary information on the population structure of *H. quadrimaculatus*. Using a mitochondrial gene region we investigated the occurrence of genetic differentiation among populations of *H. quadrimaculatus* in the Verde Island Passages.

Materials and methods

As larval Clupeidae are caught locally (within 100 km), seventy-five *H. quadrimaculatus* larvae samples were collected from fish markets across five sites in the Verde Island Passages (Table 1) and brought to the Genetic Fingerprinting Laboratory (GFL) for identification.

Tissue samples for genomic extraction were taken from the dorsal part of the lateral body wall of each individual fish. DNA was extracted from muscle tissue samples using the Chelex® (Biorad) resin beads extraction protocol (Yue and Orban, 2005). Chelex® extracts were subjected to polymerase chain reaction (PCR) using the published primers for the partial 16S rRNA gene region (Palumbi, 1996) 16Sar: 5′-CGCCTGTTTATCAAAAACAT-3′ and 16Sbr: 5′- CCG GTCTGAACTCAGATCACGT-3′. Reaction mixture of 25 µl was composed of 1 µl of DNA template, 0.5 mM of dNTP, 3.5 mM of $MgCl_2$, 0.5 mM of primers and 0.125 µl of *Taq* DNA polymerase. PCR products were amplified by initial denaturation of 95°C for 10 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 45 s, extension at 72°C for 45 s and a final extension at 72°C for 10 min. Final products were shipped to Macrogen Korea for DNA sequencing.

The 16S rRNA sequences obtained from Macrogen Korea were assembled using CODONCODE v5.6 (Biomatters, 2012) and aligned in CLUSTALX (Larkin et al., 2007). To verify the identity of the larvae, DNA sequences of Clupeidae known to be geographically distributed in the Philippines were included in the analyses (D. A. Willette, unpublished data). Phylogenetic analysis of 16S rRNA sequences was performed using the software MEGA v5 using the maximum-likelihood method with 1000 bootstrap replicates (Tamura et al., 2011).

Table 1
Sampling information for *Herklotsichthys quadrimaculatus* larvae

Location	N	Collection date	Latitude	Longitude
Batan, Batangas	22	December 2011	13.803°N	120.953°E
Calatagan, Batangas	15	January 2012	13.842°N	120.637°E
Pola, Oriental Mindoro	6	December 2011	13.148°N	121.435°E
San Juan, Batangas	26	November 2011	13.846°N	121.404°E
Tingloy, Batangas	6	January 2012	13.669°N	120.870°E
Total	75			