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An Observation on the RNA/DNA Ratio of Individual Japanese Anchovy Larva from Four Hydrographic Regions in the Northeastern Waters of Taiwan

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ABSTRACT

The RNA/DNA ratios of Japanese anchovy (*Engraulis japonica*) larvae caught from the northeastern waters of Taiwan were examined and compared in this study. Four types of water masses can be distinguished in the area; thus, four hydrographic regions are defined as the southern East China Sea (SECS), Kuroshio (KUR), surface mixing water (MIX) of SECS and KUR, and upwelling water (UWW) from subsurface KUR. The nucleic acid contents of individual larva were measured, and the RNA/DNA ratio was used as an index to compare the nutritional condition of larvae from among the waters. In general, the RNA and DNA contents were related to body size, but the RNA content had higher variability in correspondence to hydrographic differences. Larvae from the KUR showed a significantly lower RNA/DNA ratio than did those from the SECS, MIX and UWW. Finally, a relatively poor nutritional condition of Japanese anchovy larvae in the KUR is inferred, and the distribution pattern of Japanese anchovy in the waters around Taiwan is explained.

Key Words: nutritional condition, anchovy, East China Sea, Kuroshio

I. Introduction

The Japanese anchovy *Engraulis japonica* is an important pelagic species in the East China Sea (Hayasi, 1967). During spring, its southern stock migrates to the northeastern waters of Taiwan for spawning (Young and Chiu, 1994). The area, including a coastal bay area, is an important nursery area for planktonic larvae of Japanese anchovy (Huang and Chiu, 1996).

The shelf area of northeastern Taiwan is part of the East China Sea, the water of which is affected seasonally by intrusion of the Kuroshio from the southeast (Chuang and Liang, 1994). This intrusion event induces mixing of Kuroshio surface water with that of the East China Sea and causes the local Kuroshio subsurface water to move upward (Chern *et al*., 1990; Liu *et al*., 1992; Gong *et al*., 1995). The upwelling in the northeastern waters of Taiwan has been found to be rich in nutrients (Gong *et al*., 1995) and chlorophyll-*a* (Chen, 1995).

The quantity and quality of food items in a water environment are major factors influencing fish stock in their early life stage. A lack of food or a mismatch in distribution of larval fish and food organisms is, therefore, a major cause of high larval mortality (Fortier *et al*., 1995; Busch, 1996; Gotceitas *et al*., 1996). Since the RNA/DNA ratio has been determined to be an effective index of the nutritional status in the larval stage (Buckley, 1984; Clemmesen, 1994), we adopt this method to examine the RNA/DNA ratio of Japanese anchovy larvae from four hydrographic regions in the northeastern waters of Taiwan, and to infer their relative fitness in different marine environments.

II. Materials and Methods

Japanese anchovy larvae were sampled during April 24-28, 1996 by R/V Ocean Research I in the northeastern waters of Taiwan, approximately bounded by 25º-27ºN and 121º-124ºE (Fig. 1). The larvae were collected using a Bongo net (Hydro-Bios), with a mouth diameter of 60 cm, length 250 cm and mesh size 500 μm, operated in a water column within a depth of 200 m. Obtained Japanese anchovy larvae were sorted within five minutes and stored in liquid nitrogen immediately.

At each station, profiles of the temperature and salinity of the water were measured using a gauge with conductivity, temperature and depth (CTD) sensors (SBE 9/11, Sea-Bird Electronics, Inc.). The patterns of temperature-salinity diagrams, the bottom depth, and satellite images of the sea surface temperature were used to classify four hydrographic regions. These include two basic end-

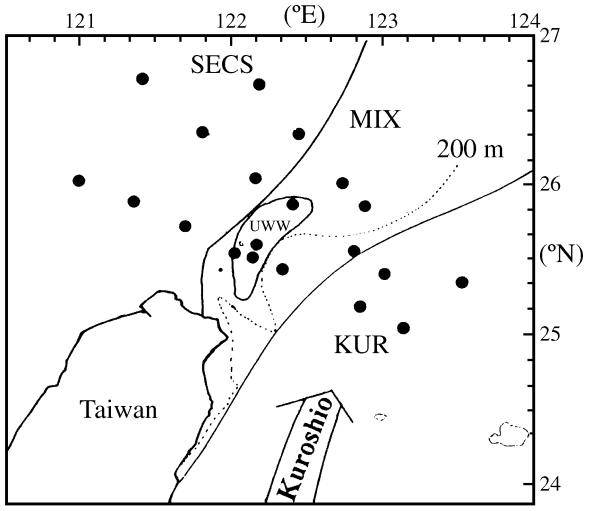

Fig. 1. Schematic diagram showing four hydrographic regions in the waters off northeastern Taiwan: the southern East China Sea (SECS), the mixing water (MIX), the upwelling water (UWW) and the Kuroshio (KUR). Solid circle (•): Survey stations occupied.

members of the southern East China Sea (SECS, low temperature and low saline situated on the continental shelf) and the Kuroshio (KUR, high saline and high temperature situated far offshore, with a bottom much deeper than 500 M), and two derived members of the mixing water (MIX, relatively low surface temperature situated on the continental slope between the SECS and KUR) and the upwelling water (UWW, surface temperature less than 20 ºC, forming a significant cold dome in satellite images). The identification of water categories was carried out by generally following Chiu and Lee (1991), Chiu and Hsyu (1994), and Huang and Chiu (1998). For each larva, the body length was measured using the notochord length (pre-flexion and flexion larvae) or total length (post-flexion larvae). Measurements of RNA and DNA contents basically followed Clemmesen (1993), with minor modification. The RNA and DNA were first purified from individual larva homogenate, and the contents were subsequently measured using the fluorescence photometric method with the nucleic acid fluorescence dye, ethidium bromide. The content of total nucleic acid (RNA+DNA) was determined first, and the content of DNA was determined after RNA having been digested by RNase. The fluorescence index was read by using a spectrophotometer (F-2000, Hitachi), where a wavelength of 365 nm was selected for excitation, and the emitted light was observed at 590 nm.

Thirty individuals from each classified region were randomly selected for this study. Since the RNA/DNA ratio may be confounded by the body size (Westerman and Holt, 1988), analysis of variance was used to separate the size effect, and comparison of the ratios among different waters was carried out within a relatively small body size interval of 2 mm total length (TL). Duncan's multiple range test (Snedecor and Cochran, 1980) was used to test the differences in the RNA/DNA ratios among the 4 regions.

III. Results

1. General Status

During the spring of 1996, four water types, *viz*. SECS, MIX, UWW and KUR, were identified in the northeastern waters of Taiwan (Fig. 1). The environmental factors pertaining to the stations from these waters, including bottom depth, water temperature and salinity, were measured at a depth of 50 m, and the results are shown in Table 1. The SECS, located on the continental shelf, had a shallower bottom depth of 67-128 m, a temperature of 15.86-20.11 ºC and a salinity of 33.49-34.25 ppt. The MIX, lying on the borders of the continental shelf, had an intermediate bottom depth of 113-709 m, a temperature of 18.46-20.41 ºC and a salinity of 34.42-34.56 ppt. The UWW was discovered in a relatively smaller range with a depth of 97-194 m, a temperature of 17.71-18.84 ºC and a salinity of 34.40-34.51 ppt. The temperature of the UWW was lower than that of the water in its vicinity although the extreme cold water found in the SECS were located very close to the coast of Mainland China with no ambiguous overlap in geographic range. Therefore, the UWW can be identified reasonably precisely. The KUR, which stretched out into the open sea, had a very deep bottom of 839-1343 m, hot water in the range 21.61-24.49 ºC and a high level of salinity of 34.55-34.81 ppt.

2. Variation in RNA/DNA Ratio with Body Size

The scatter plots of DNA and RNA contents for each larva are shown in Fig. 2. The magnitude of the RNA content per individual larva apparently increased with the increase in fish size, but that of DNA remained fairly constant. A linear relationship between body length and RNA was found as follows: RNA (μg/ind.) = –10.291 ± 1.992 TL (mm) (r = 0.828, p < 0.001, n = 120). The RNA/DNA ratio was also found to be body size dependent (Fig. 3). The lowest

Table 1. Basic Information of Four Hydrographic Regions where Japanese Anchovy Larvae Were Sampled

Hydrographic	Environmental factor (ranges)		
Regions	Depth (m)	Temperature (ºC)[a]	Salinity (ppt)[a]
SECS	67 – 128	15.86 – 20.11	33.49 – 34.25
MIX	113 – 709	18.46 – 20.41	34.42 – 34.56
UWW	97 – 194	17.17 – 18.84	34.40 – 34.51
KUR	839 – 1343	21.61 – 24.49	34.55 – 34.81

[a] measured at a depth of 50 m.

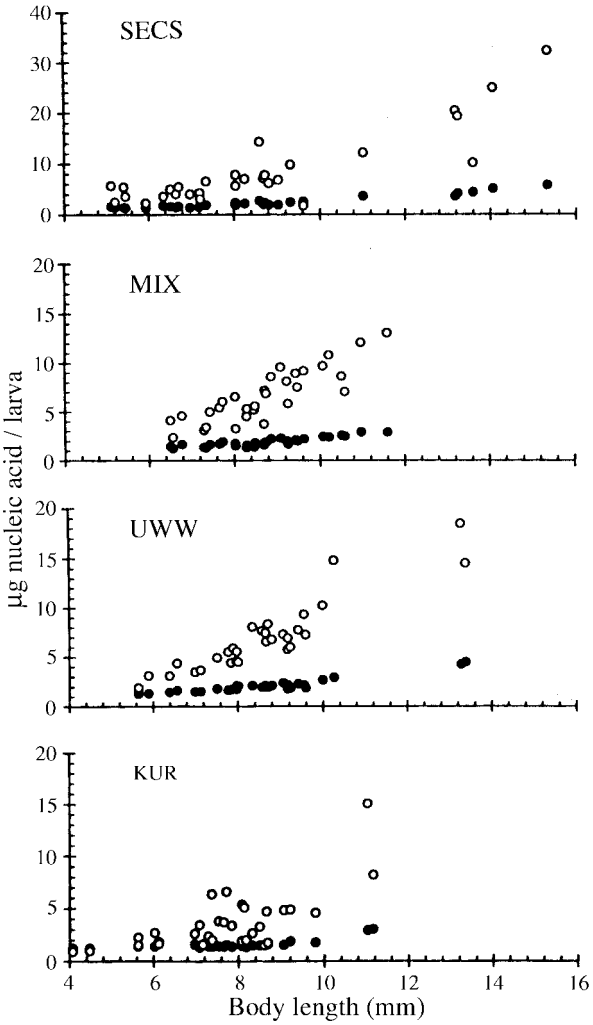

Fig. 2. Scatter plots showing RNA (◦) and DNA (•) contents (μg) and total length (mm) of individual Japanese anchovy larva.

value of the ratio (2.166 ± 0.176) was found for fish with 5.0-6.9 mm TL. The ratio increased as the size of the fish increased: 2.872 ± 0.115 (7.0 – 8.9 mm TL), 3.550 ± 0.165 (9.0 – 10.9 mm TL), 3.911 ± 0.554 (11.0 – 12.9 mm TL), and 4.409 ± 0.469 (13.0 – 14.9 mm TL), for each size group, respectively.

3. Variation in RNA/DNA Ratio with Hydrographic Region

The ratios for fish obtained from four hydrographic regions were found to be different (Fig. 4; $p < 0.001$). Multiple range test revealed a group of SECS + MIX + UWW to be in contrast to KUR. The average ratios and standard deviation were 3.259 ± 0.213, 3.388 ± 0.130, and 3.180 ± 0.144, for SECS, MIX and UWW, respectively. However, they were found to be significantly lower for KUR (2.181 ± 0.204).

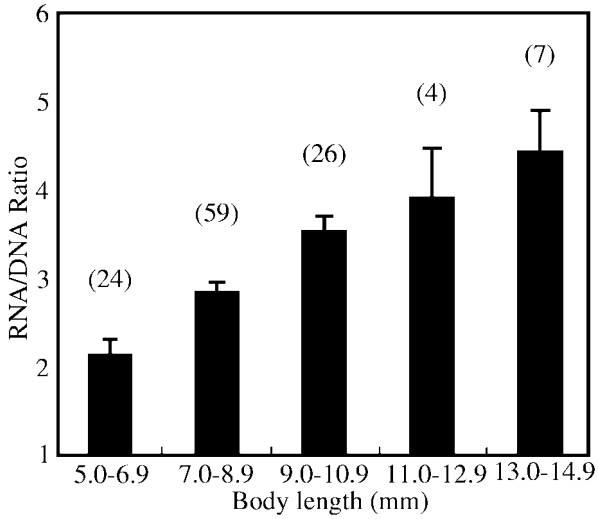

Fig. 3. Plot of RNA/DNA ratio by body length (mm) for Japanese anchovy larvae. Vertical bar, within group standard deviation; number in (), number of larva examined.

IV. Discussion

Upwelling and intrusion are the two mechanisms which transport water from the Kuroshio onto the southern East China Sea shelf of northeastern Taiwan. The upwelling Kuroshio water (UWW) carries surplus nutrients to the photosynthetic layer of the shelf water and causes high primary productivity in the northeastern waters of Taiwan (Gong *et al*., 1995). The intrusion of the Kuroshio into the southern East China Sea causes water mixing and forms an ecotonal region (MIX) between the southern East China Sea and the Kuroshio. Therefore, four hydrographic regions and, thus, ecological regions can readily be found during the spring blooming season of Japanese anchovy larvae.

It has been suggested that fish production is high in upwelling areas (Ryther, 1969). However, this phenomenon did not occur in the core upwelling region in the waters of northern Taiwan (Chu *et al*., 1993). This study is the first time to compare the nutritional condition of anchovy larvae across four hydrographic regions in the waters of NE Taiwan. In our study, no high values of the RNA/DNA ratio, an index of nutritional condition (Canino, 1994), were found for larval Japanese anchovy from the UWW (Fig. 4). This result further shows that the persistent upwelling water of northeastern Taiwan might not provide additional food to Japanese anchovy larvae. Probably, this further shows that the upwelling water has little benefit for fish species of non-phytoplankton eaters other than Japanese anchovy. However, this inference should not be extended prematurely to other fish larvae, such as scombers (*Auxis* spp.) and scads (*Decapterus* spp.), which have been found to be abundant in the UWW (Huang and Chiu, 1998).

The migration of adult Japanese anchovy to the coast

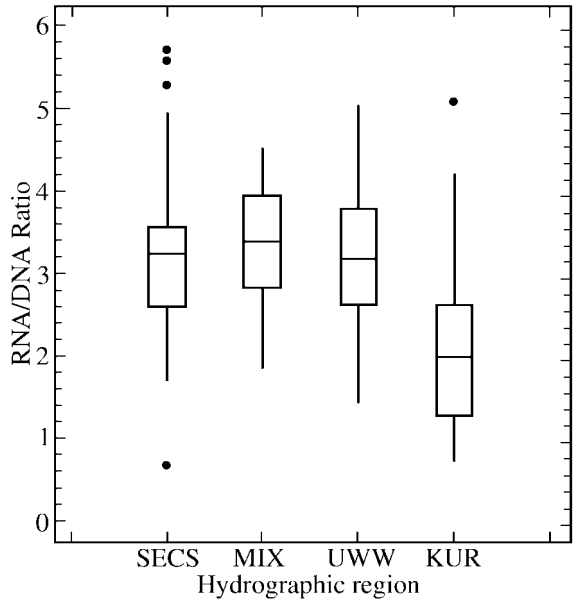

Fig. 4. Box-and-whisker plots showing the variation of the RNA/DNA ratio of Japanese anchovy larvae among four hydrographic environments. Bar within box, group median; box, range of 50% individuals; whiskers, the extremes of data; four outliers (•) observed in SECS and one in KUR.

of Taiwan is related to reproduction (Young and Chiu, 1994). The earliest larvae were found in I-lan Bay and Pan-Cha-Yu, adjacent to the UWW location (Chiu *et al*., 1997). Relatively few larvae had been transferred across the hydrographic front into the KUR (Young *et al*., 1995). The RNA/DNA ratios of the anchovy larvae collected in the KUR were the lowest in the studied hydrographic regions (Fig. 4). Based on assessment using the height of midgut cells, Hung (1995) found that 75% of the anchovy larvae in the Kuroshio were starving. Shimizu *et al*. (1989) also showed that the nutritional condition of the sardine *Sardinops melanostictus* collected from the offshore waters of the Kuroshio is poorer than that of the same sardine collected from the coastward side. It is, hence, hypothesized that the oligotrophic Kuroshio water along the western boundary of the Pacific is incapable of supporting anchovy larvae with sufficient quantity and suitable quality of food. On the other hand, the better food quality found in non-KUR regions, as indicated by an increase of 20-40% in the protein level (Chen and Chen, 1992), greatly enhances the nutritional conditions for Japanese anchovy. As a result, the Kuroshio water is not a favorable environment for the development of Japanese anchovy larvae. Accordingly, no adult Japanese anchovy were found in the Kuroshio and the offshore area of eastern Taiwan (Young *et al*., 1994).

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臺灣東北水域日本鯷仔魚個體RNA/DNA比值的考察

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摘　要

本研究對取自臺灣東北水域日本鯷(*Engraulis japonica*)仔魚的個體RNA/DNA比值進行考察，並比較其在不同水文區域間之差異。臺灣東北水域有四個水文區域：東海南部水區(SECS)、黑潮水區(KUR)、源自黑潮及東海表層之混合水區(MIX)及由黑潮次表層上升的湧升水區(UWW)。本考察先測量單尾日本鯷仔魚之核酸含量，再計算其RNA/DNA比值，以此為指數比較不同水文區域間之營養差異。一般而言，日本鯷仔魚的個體RNA及DNA之含量與體型大小有關，但RNA含量的變異度(variability)較高；此情形對應著來自不同水文區域的環境屬性。來自KUR的仔魚其RNA/DNA比值較來自SECS、MIX和UWW者為低；因此可據之推論位於黑潮水區內的日本鯷仔魚營養狀態較差。此結果恰可解釋臺灣附近水域的黑潮水區及東部水域不為日本鯷成魚分布的原因之一。