Indirect Evidence on Sex Reversal with Sex Ratio of
*Tegillarca granosa* (Bivalvia: Arcidae) and
*Ruditapes philippinarum* (Bivalvia: Veneridae)

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ABSTRACT: This study attempts to propose the possibility of the sex reversal in *Tegillarca granosa* and *Ruditapes philippinarum* by confirming the changes in the sex ratio with the shell length (SL) in the same population level. For analysis of sex ratio with SL, 1500 individuals of *T. granosa* (SL 10.1-45.0 mm) and 712 individuals of *R. philippinarum* (SL 15.1-70.0 mm) were used. Sex was analyzed histologically. The average sex ratios (F:M) of *T. granosa* and *R. philippinarum* were 1:1.22 and 1:0.96, respectively. However, sex ratio was found to differ when the clams were divided into groups according to SL in 5.0 mm intervals. Both species displayed the tendency of increase in the proportion of female with increase in SL. In this study, changes in the sex ratio in accordance with the growth of *T. granosa* and *R. philippinarum* are determined to be indirect evidence that signifies their sex reversal.

Key words: *T. granosa*, *R. philippinarum*, Sex ratio, Sex reversal

INTRODUCTION

Sex of bivalves is classified into gonochorism and hermaphroditism, and hermaphroditism is further divided into synchronous (simultaneous or functional) and asynchronous (sequential) hermaphroditism. The asynchronous hermaphroditism signifies sex reversal in accordance with seasons, resultantly (Heller, 1993; Gosling, 2004).

Indirect evidence of sex reversal in bivalves is a change in the sex ratio of a population with size (Orton, 1933; Galtsoff, 1964; Guo et al., 1998; Eversole, 2001; Gosling, 2004). Sex reversal of bivalves has been reported in the Ostreidae (Orton, 1933; Galtsoff, 1964; Thompson et al., 1996; Guo et al., 1998; Gosling, 2004), Pectinidae (Osanai, 1975; Ventilla, 1982) and Veneridae (Eversole, 2001). Another indication of indirect evidence is the fact that large number of bivalves have an inactive stage during the reproductive cycle and classification of morphological sex is impossible as the gonad tissue is completely degenerated during this period (inactive stage) (Chung et al., 1994; García-Domínguez et al., 1994; Behzadi et al., 1997; Lee, 1997; Villalejo-Fuerte & García-Domínguez, 1998; Park et al., 2003; Wi et al., 2003). In the case of these bivalves, there is possibility that they may develop as different sex when the next reproductive cycle begins.

This study attempted to present indirect evidence on the sex reversal of *Tegillarca granosa* and *Ruditapes philippinarum* by examining the changes in sex ratio with shell length and also to suggest basic information to definitively investigate their sex.

MATERIALS AND METHODS

1. Materials

*Tegillarca granosa* used for sex ratio analysis was collected from the Jangsu Bay of Yeosu on the southern
coast of Korea from April 2007 to June 2011. Total number of *T. granosa* used for sex ratio analysis was 1,500 (shell length 10.1-45.0 mm) (Table 1). *Ruditapes philippinarum* used for sex ratio analysis was collected from the intertidal zone of Goheung on the southern coast of Korea from June 2009 to August 2011. Total number of *R. philippinarum* used for sex ratio analysis was 712 (shell length 15.1-70.0 mm) (Table 2).

2. Histological Analysis

Histological techniques were also used to confirm the sex and gonadal development of each specimen. Specimen preparation for light microscopy was performed according to the methodology of Drury & Wallington (1980). The study clams were dissected, and their visceral mass, which included the gonad, was fixed in aqueous Bouin’s solution for 18 h. and rinsed in running water for 24 h. and then dehydrated through a graded ethanol series (70-100%). The tissues were then embedded in paraplast (McCormick, USA). Embedded tissues were sectioned at 4-6 um thickness using a microtome (RM2235, Leica, Germany). Samples were stained with Mayer's hematoxylin-0.5% eosin (H-E).

3. Statistical Analysis

<table>
<thead>
<tr>
<th>Shell length (mm)</th>
<th>Number</th>
<th>Sex ratio (F:M)</th>
<th>$\chi^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>10.1-15.0</td>
<td>20</td>
<td>2</td>
<td>18</td>
<td></td>
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<td>15.1-20.0</td>
<td>75</td>
<td>25</td>
<td>50</td>
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<td>20.1-25.0</td>
<td>73</td>
<td>23</td>
<td>50</td>
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<td>25.1-30.0</td>
<td>537</td>
<td>219</td>
<td>318</td>
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<td>30.1-35.0</td>
<td>598</td>
<td>277</td>
<td>321</td>
<td></td>
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<td>35.1-40.0</td>
<td>175</td>
<td>118</td>
<td>57</td>
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<td>40.1-45.0</td>
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<td>13</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1500</td>
<td>677</td>
<td>823</td>
<td>1:1.22</td>
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<table>
<thead>
<tr>
<th>Shell length (mm)</th>
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<th>Sex ratio (F:M)</th>
<th>$\chi^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
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<td>2</td>
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<td>-</td>
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<tr>
<td>20.1-25.0</td>
<td>69</td>
<td>29</td>
<td>40</td>
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<td>4</td>
<td>2</td>
<td>1:0.50</td>
</tr>
<tr>
<td>Total</td>
<td>712</td>
<td>364</td>
<td>348</td>
<td>1:0.96</td>
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</table>
Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Microsoft Co. WA). Sex ratio was assessed by Chi-square ($\chi^2$) test. In all cases significance was established at $p<0.05$.

**RESULTS**

1. Sex Ratio

1) *Tegillarca granosa*

Sex ratio (F:M) for the entire population of *T. granosa*, shell length (SL) 10.1-45.0 mm (n=1500) was 1:1.22. However, sex ratio was found to differ when the clams were divided into groups according to SL in 5.0 mm intervals. Sex ratio displayed the results of the tendency of increase in the proportion of female as SL increased in SL 10.1-35.0 mm but increase in the proportion of male in above 35.1 mm in SL (Table 1).

2) *Ruditapes philippinarum*

Sex ratio (F:M) for the entire population of *R. philippinarum*, SL 15.1-70.0 mm (n=712) was 1:0.96. However, sex ratio was found to differ when the clams were divided into groups according to SL in 5.0 mm intervals. Although there was tendency of increase in the proportion of female as SL increased in SL 15.1-60.0 mm, increase in the proportion of male in above 60.0 mm in SL was observed (Table 2).

2. Gonadal Histology

The sexes of the study clams were separate. Both ovary and testis were composed of many gametogenic follicles, and it was possible to observe the developmental process of the germ cells within the follicle. The developmental stages of gonads were divided into five stages of inactive, early active, late active, ripe and degenerative stage. From the result of histological analysis, histological characteristics of the gonad that illustrate the process of sex reversal were not observed at either of the reproductive season and at the inactive stage (Figs. 1 and 2).

**DISCUSSION**

There is still a paucity of data on sex differentiation and sex reversal in many bivalves, which makes sex identification of some species difficult. In general, in the case of gonochorism, manifestation of sex as male or female, after having identified the morphological sex of bivalve, is limited to a prescribed time period of the life history of the given individual. In order to accurately identify their sex, continuous tracing of changes in the sex by tagging the sex of each individual is necessary. However, this is also problematic since classification of bivalves into males or females is difficult macroscopically (Coe, 1943).

There was a case in which sexual dimorphism in bivalves was reported in *Dysnomia capsaeformis* and *D. brevidens* (Mackie, 1984). However, since it is difficult to distinguish sex macroscopically, histological analysis of gonads remains the only conclusive method. However, this method cannot be carried out without killing the organism (Gosling, 2004).

Although the sex of the majority of bivalves cannot be identified externally, the ratio of females to males is the same and they are generally gonochoristic (Gosling, 2004). Hermaphroditism (synchronous or asynchronous) in bivalves was recorded in 13 families among 117 analyzed families (Heller, 1993).

Synchronous hermaphroditism is the simultaneous release of eggs and sperm by one organism during the same season. Asynchronous hermaphrodities function first as one sex, then as another. Asynchronous hermaphrodities can be protandrous, protogynous or can alternate sexuality. In protandry, the individual assumes the sex of male initially and then sex is reversed to female. The opposite happens in the case of protogyyny. Alternating sexuality is the functioning of an organism first as one sex then as another, repeatedly (Heller, 1993).

Synchronous hermaphroditism in bivalves has been reported in *Anodonta grandis* (Van Der Schalie & Locke,
1941) and *Elliptio* (Heard, 1979) of Unionidae; *Argopecten irradians* (Sastry, 1979), *Chlamys opercularis* (Sastry, 1979) and *Pecten latiauritus* (Mackie, 1984) of Pectinidae; *Fulvia mutica* of Cardiidae (Chang & Lee, 1982); *Corbicula fluminea* of Corbiculidae (Britton & Morton, 1982); and *Gemma* spp. of Veneridae (Mackie, 1984).

Asynchronous hermaphroditism in bivalves has been reported in *Crassostrea rivularis*, *C. madrasensis*, *Saccostrea glomerata* and *S. cucculata* (Asif, 1979), *Ostrea edulis* (Sastry, 1979; Gosling, 2004), *C. virginica* (Mackie, 1984; Gosling, 2004) of Ostreidae and *Mercenaria mercenaria* (Sastry, 1979; Gosling, 2004) of Veneridae.

Although sex reversal has been reported for other bivalves, such as *Patinopecten yessoensis* (Osanai, 1975; Ventilla, 1982), sex reversal studies have traditionally been conducted on oysters. Historically, the literature points to the higher proportion of males at the early stage, with increasing proportion of females arising through sex reversal at older stages.
stages of oysters (Orton, 1933; Coe, 1934; Galtsoff, 1964; Thompson et al., 1996; Guo et al., 1998).

European oyster, *O. edulis* exhibits between 10-16% male to female sex reversal in the first year, and approximately 50% male to female sex reversal in the second year, respectively (Orton, 1933). They mature as males initially and undergo sex reversal into females after the first discharge of sperm, while sex reversal is repeated throughout their life cycle (Walne, 1974). Quahog clam, *M. mercenaria*, become sexually mature at less than one year old, developing first as males but changing to an equal sex ratio in the second year (Menzel, 1989).

Sex of *T. granosa* has been reported to be gonochoristic (Lee, 1997). Chung et al. (1994) also reported the sex of *R. philippinarum* to be gonochoristic. However, in this study, sex reversal is forecasted for *T. granosa* and *R. philippinarum* based on the results of analysis of changes in the sex ratio with shell sizes. Therefore, the possibility that the sex of these species is asynchronous hermaphroditism is very high.

These conclusions on the sex of *T. granosa* (Lee, 1997) and *R. philippinarum* (Chung et al., 1994) may have been reached because of the limited investigation undertaken by these authors some stage of the life cycle of *T. granosa* and *R. philippinarum* rather than continuous tracking of the reproductive life-history over a long period.

*Crassostrea virginica* is a protandric species with an increase in the proportion of females through sex reversal as it ages. Sex reversal normally occurs when the gonad is undifferentiated between spawning seasons (Thompson et al., 1996). Although the sex of the scallop *P. yessoensis*, is male during the young stage of less than one year, sex is thereafter reversed to female. Histological analysis showed that their sex is not reversed during the inactive stage of their reproductive cycle, but is rather reversed through continuous stages during the reproductive cycle (Osanai, 1975).

From the results of Lee (1997), Chung et al. (1994) and the results of this study, which histologically analyzed the gonad through all the stages of the reproductive cycle of *T. granosa* and *R. philippinarum*, no observation could be made of the histological characteristics of the gonad that illustrated the process of sex reversal at any stage of the reproductive cycle. Therefore, it is determined that the sex reversal in the study clams occurs during the inactive stage following the spawning season.

Two factors, namely genetic and environmental, are involved in the changes in sex ratio and sex determination of bivalves. In the case of *Pomacea canaliculata*, *C. gigas* and *Mytilus* sp., an oligogenic sex determination method was used, which is a mechanism that results in highly diversified sex ratio and sex determination by small number of genes (Guo et al., 1998; Yusa, 2007). Factors such as temperature, food availability and day length are involved in environmental sex determination of bivalves (Yusa, 2007). Changes in sex ratio due to exposure to pollutants, such as EDCs and heavy metals during the inactive season, have also been reported in *M. arenaria* (Gagné et al., 2005), *Gomphina veneriformis* (Lee & Park, 2007; Ju et al., 2009) and *Scapharca broughtonii* (Lee et al., 2009).

As the result of this study, although the sex reversal of *T. granosa* and *R. philippinarum* is predicted, additional researches are necessary for presentation of direct evidences and factors of sex reversal in these species.

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