Survival of the Ark Shell, *Scapharca subcrenata* and Physiological and Histological Changes at Decreasing Salinity

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We examined physiological and histological responses related to the survival, oxygen consumption, excretion, and O/N ratio of the ark shell, *Scapharca subcrenata*, as a result of salinity changes. The 20-day LS_{50} (median lethal salinity) at 15°C was 13.87 practical salinity units (psu; confidence limits 10.30-18.74 psu), whereas the 14-day LS_{50} at 25°C was 12.59 psu (confidence limits 8.03-18.16 psu). In conditions of decreasing salinity, the osmolarity of individuals acclimated within 5 h above 26.4 psu but required more than 60 h below 13.2 psu. Oxygen consumption and ammonia excretion rates varied irregularly as salinity decreased. The O/N ratio was 19 and 27 at water temperatures of 15°C and 25°C, respectively, but decreased to 1-10 as salinity declined. The effects of decreasing salinity were observed in the histological changes to each organ of *S. subcrenata*. As salinity decreased, cilia fell off, the epithelial layer underwent necrosis and vacuolation, the connective tissue layers of the mantle and visceral mass were destroyed, and hemocytes increased in the gills. The results of this study could prove important in investigating causes of mass mortality and managing shellfish aquaculture farms.

Key words: *Scapharca subcrenata*, Salinity, Survival, Physiological change

Introduction

Aquaculture in Korea has grown rapidly over the last several decades (FAO, 2006) and provides important sources of protein. However, in tandem with industrial development, most coastal aquaculture farms are experiencing reduced yields due to mass mortalities of cultured species. Mass mortalities most often occur in the summer, when water temperature increases, and localized torrential rainfalls occur. *Scapharca subcrenata*, a bivalve of the Arcidae family, is a cultured shellfish that lives in muddy waters at a depth of 4-6 m. Yeoja Bay in Jeonnam is the largest aquaculture location (Min, 2004). Ark shell production in Korea reached 3,842 MT in 2001 but dropped to 2,440 MT in 2003 and has undergone cyclic variations since then, yielding 10,849 MT in 2004, 5,063 MT in 2006, and 1,637 MT in 2008 (Korea National Statistical Office, 2009).

Shellfish cultivation is potentially one of the most sustainable forms of mariculture, requiring no artificial food inputs, as the animals obtain all of their nutrition from phytoplankton, microphytobenthos, and organic detritus (Cranford and Grant, 1990; Grant, 1999; Scholten and Smaal, 1999; Hawkins et al., 2001). Nevertheless, the rapid growth of the industry has inevitably raised sustainability issues (Grant, 1999). Cultivated bivalve filter feeders play a key role in many coastal ecosystems, owing to their high filtration capacity and culture density (Smaal et al., 2001). However, as more living biomass accumulates, the proportion of primary production that is available for further growth of bivalve biomass declines (Dame and Prins, 1998), and factors such as increased biodeposition may contribute to significant environmental changes, such as sediment anoxia (NRCA, 1998).

The physiological responses of organisms represent the sum of all of their cellular and biochemical reactions to environmental influences. Thus, indivi-

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dual organisms may reflect environmental deterioration before its effects are manifest in the population or community as a whole (Bayne, 1985). Among external environmental factors affecting shellfish, water temperature has perhaps the most profound effect, affecting not only metabolic rate but also activity level and energy balance (Newell and Kofoed, 1977). Salinity changes disrupt the uniformly balanced state between water content and the influx/efflux of salts in cells. Rapid changes in salinity cause shellfish to close their shells and reduce metabolism to minimize the effect of the metabolic rate reduction on cellular volume (Lange, 1972). The compound effect of water temperature and salinity has an enormous impact on the stability of shellfish production yields.

This study examines the physiological effects of sudden changes of water temperature and salinity on the respiratory, excretory, and histological stress responses of the ark shell S. subcrenata.

Materials and Methods

Sample collection

S. subcrenata samples were collected from Yeoja Bay near Yeosu, Chonnam Province, South Korea, from March to November 2007 (Fig. 1), stocked in a 5-ton saltwater tank in the laboratory, and used in experiments after 2-week periods of acclimation to experimental water temperatures. Samples were cleaned of epibionts and fed a mixture of Isochrysis galbana, Tetraselmis sp., and Chaetoceros sp. during the experimental period.

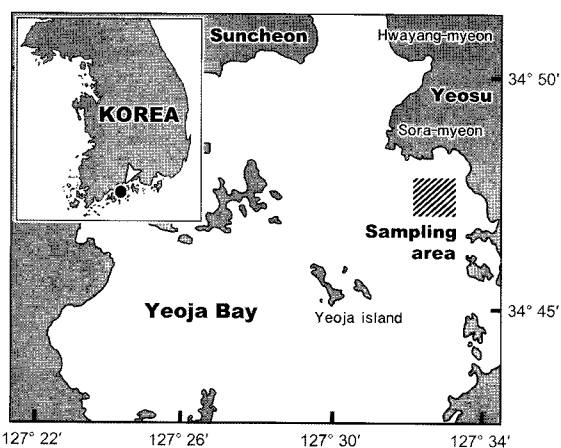Fig. 1. Sampling area of ark shell, Scapharca subcrenata.

Survival and physiological parameter measurements

We established 12 experimental regimes (salinity: 3.3, 6.6, 13.2, 19.8, 26.4, and 33.0 psu; seawater temperature: 15°C and 25°C), with 33.0 psu used as the control. Salinity was measured using an automatic salinity gauge (PR-100SA; Atago, Japan). LS_{50} (median lethal salinity) was calculated using the probit method of Finny (1971), with the histological responses of test animals transferred suddenly to higher or lower salinity analyzed. Oxygen consumption rates were determined using an oxygen meter (Orbis 3500 analyzer, neuchatel/geneva-switzerland), and ammonium was measured simultaneously with oxygen consumption. Two-mL water samples taken from each respiration chamber were immediately analyzed using the phenolhypochloride method (Solorzano, 1969). To measure osmolarity, blood was withdrawn from the heart area of S. subcrenata specimens with a 1-mL intravenous syringe and analyzed by osmometer (Osmomat 030; Genotec GmbH, Germany). The atomic ratio of oxygen to nitrogen (O/N) was calculated based on oxygen uptake and ammonium nitrogen excretion and expressed in atomic equivalents according to the formula $O/N=(mg\ O_2/h/16)/(mg\ NH_4-N/h/14)$ (Widdows and Johnson, 1988). To measure the specimens, the shells were removed. The average wet weight and shell length of the S. subcrenata specimens were 3.3 ± 0.71 g and 34.1 ± 2.6 mm, respectively. After dissection of the mantle, gill, and visceral mass, the tissues were fixed in aqueous Bouin's solution for 24 h and rinsed in running tap water for 24-36 h. The preparations were embedded in paraplast (McCormick, USA) after dehydration through a graded ethanol series, as described by Drury and Wallington (1980). The embedded tissues were sectioned at 4-5 µm thickness using a microtome (RM2235, Leica, Germany). The tissues were stained with Mayer's hematoxylin-0.5% eosin (H-E) stain and observed under a light microscope (BX50, Olympus, Japan).

Results

Survival

To measure the salinity tolerance of the ark shell S. subcrenata, we measured the survival rate of specimens exposed to each experimental salinity level for 20 or 14 days at 15°C or 25°C. At 15°C, the survival rate of S. subcrenata exposed to more than 19.8 practical salinity units (psu) was 100% throughout the exposure period. However, at less than 13.2 psu, the survival rate dropped rapidly after 15 days (Fig. 2), resulting in a 20-day LS_{50} of 13.87 psu (confidence limit 10.30-18.74 psu; Table 1). However, at 25°C, the survival rate at less than 6.6 psu was less than

Table 1. Salinity tolerance (LS_{50}) on each specific period in several cultured bivalves
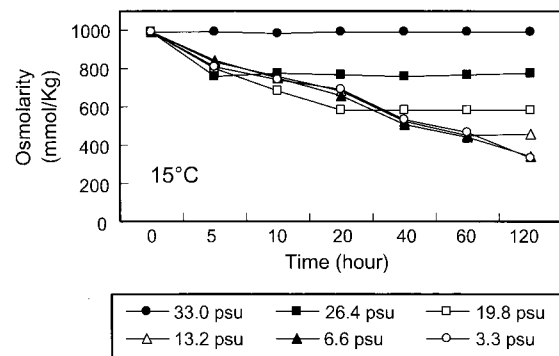
Species	Individual size	Acclimation Temperature (°C)	Exposure period (day)	LS_{50} (psu)	References
Mytilus coruscus	Juvenile	20	9	17.0-21.8	Shin and Wi (2004)
Ruditapes philippinarum	Juvenile	18	12	23.0-24.9	Shin et al. (2000)
Haliotis diversicolor	Juvenile	20	-	20-45	Chen and Chen (2000)
Argopecten purpuratus	Shell height, 25-100 mm	12	-	27.0	Navarro and Gonzalez (1998)
Scapharca broughtonii	Adult	25	9	16.5 (14.9-18.1)	Shin et al. (2006)
Tegillarca granosa	Adult	25	11	16.8 (12.9-21.2)	Moon (2005)
Anadara granosa	Adult	28-30	7	19.0	Davenport and Wong (1986)
Scapharca subcrenata	Adult	25	14	12.5	this paper
Scapharca subcrenata	Adult	15	20	13.8	this paper
Tresus keenae	Juvenile	20	5	29.1	Shin and Yang (2005)
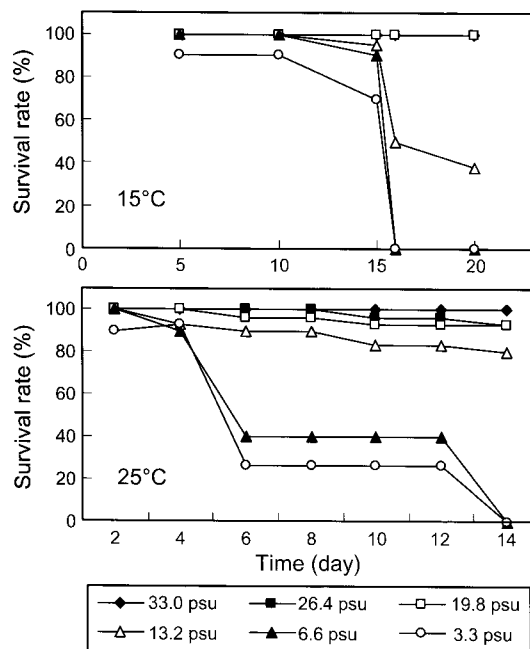
Fig. 2. Survival rate of ark shell, *Scapharca subcrenata*, exposed to different salinity regimes at 15 and 25°C.

Fig. 3. Osmolarity of ark shell, *Scapharca subcrenata* exposed to different salinity at 25°C.

40% after 6 days exposure, and by day 14 of this regime, all of the remaining animals had died. On the other hand, at more than 13.2 psu, the survival rate was greater than 80% throughout the exposure period (Fig. 2), resulting in a 14-day LS_{50} of 12.59 psu (confidence limit 8.03-18.16 psu; Table 1).

Osmolarity

Fig. 3 illustrates changes of *S. subcrenata* osmolarity in relation to salinity reduction over exposure time. At 26.4 psu, osmolarity was 773 osmol/kg, and the specimens acclimated to the osmolarity of the surrounding seawater within 5 h of exposure. However, at 19.8 psu, osmolarity was 580 osmol/kg, and acclimation required 20 h. At 13.2 psu, osmolarity

decreased gradually, and specimens acclimated to the surrounding seawater at hour 60 at 456 osmol/kg. At less than 6.6 psu, osmolarity decreased continuously until 120 hr, at which point acclimation had not been achieved (Fig. 3).

Oxygen consumption

The oxygen consumption rates of *S. subcrenata* groups exposed to 33.0 psu at 15°C and 25°C were 0.21-0.23 mg and 0.35 mg O_2/g dry wt/h, respectively (Fig. 4). At 15°C, the oxygen consumption rate of *S. subcrenata* at less than 33.0 psu was lower than that of the 33.0 psu group throughout the experimental period. At 26.4 psu at 15°C, the oxygen consumption rate was 0.16 mg O_2/g dry wt/h on day 10 and 0.18 mg O_2/g dry wt/h on day 20 of exposure, which is 14.1-30.0% lower than that of the 33.0 psu group (Fig. 4). At 19.8 psu at 15°C, the oxygen consumption rate was 0.18 mg O_2/g dry wt/h on day 20 of exposure, which is 12.4-19.8% lower than that of the 33.0 psu group. At less than 6.6 psu, the oxygen consumption rate was 0.04-0.06 mg O_2/g dry wt/h, which is a substantial (70.9-79.7%) reduction from that of the 33.0 psu group, and physiological vitality also decreased substantially. On the other hand, at 25°C,
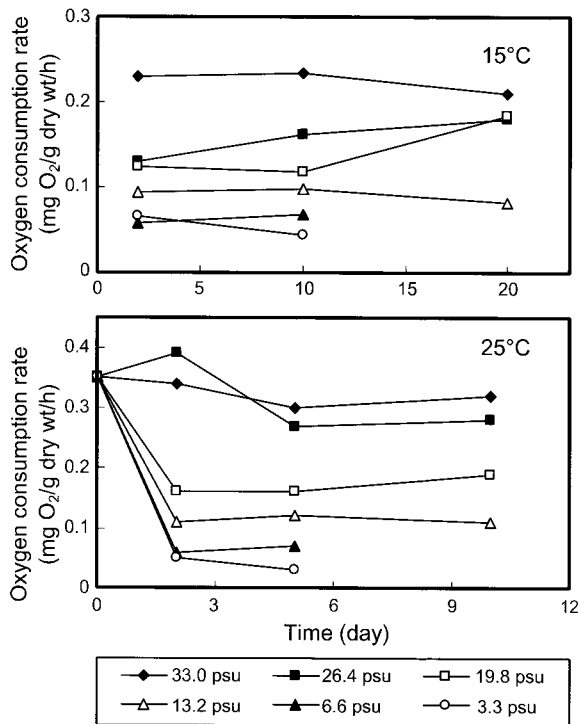
Fig. 4. Oxygen consumption rate of ark shell, *Scapharca subcrenata*, exposed to different salinity at 15 and 25°C.

the oxygen consumption rate of *S. subcrenata* at 26.4 psu slightly increased to 0.39 mg O_2/g dry wt/h on day 2 of exposure, but then decreased to 0.28 mg O_2/g dry wt/h on day 10, which is 10.6-12.5% less than that of the 33.0 psu group from day 2 of exposure (Fig. 4). At less than 6.6 psu, the oxygen consumption rate was a very low 0.07-0.03 mg O_2/g dry wt/h, which is 76.6% less than that of the 33.0 psu group (Fig. 4).

Ammonium excretion

The ammonium excretion rate of the 33.0 psu group was 10.1-11.1 µg NH_4-N/g dry wt/h. and 36.6-40.1 µg NH_4-N/g dry wt/h at 15°C and 25°C, respectively. Although the ammonium excretion rate increased initially during exposure to lower salinity, it decreased as exposure was prolonged. At 15°C, the excretion rate was 13.3 µg NH_4-N/g dry wt/h on day 10 of exposure to 26.4 psu, but it decreased to 9.39 µg NH_4-N/g dry wt/h on day 20 (Fig. 5, upper panel). At less than 6.6 psu, the excretion rate decreased to 6.18-1.59 µg NH_4-N/g dry wt/h on day 10. At 25°C, the ammonium excretion rate of the ark shells was highest on day 5 at more than 13.2 psu (excluding the 33.0 psu group), but then decreased rapidly by day 10 (Fig. 5, lower panel).
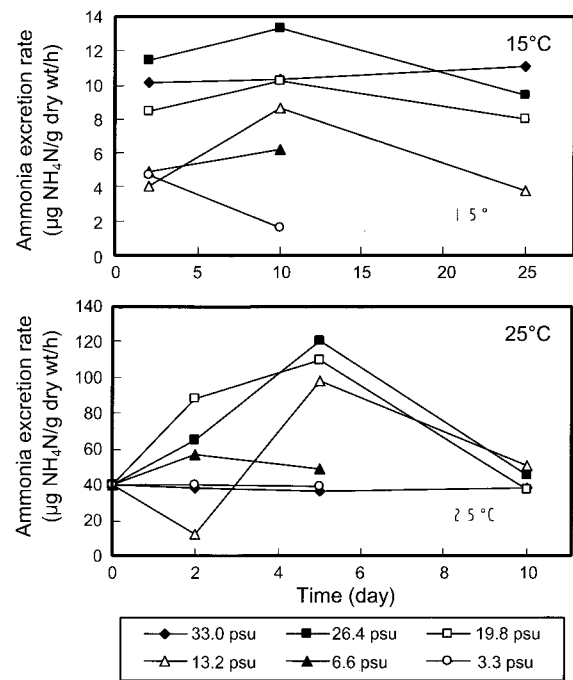

Fig. 5. Ammonia excretion rate of ark shell, *Scapharca subcrenata*, exposed to different salinity at 15 and 25°C.
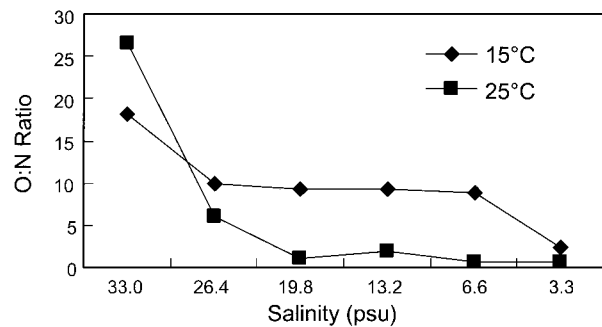

Fig. 6. O/N ratio changes of ark shell, *Scapharca subcrenata*, with different salinity at 15 and 25°C.

O/N ratio

The O/N ratio of *S. subcrenata* differed with the salinity (Fig. 6). At 33.0 psu, the O/N ratio was 19 at 15°C and 27 at 25°C. When ark shells were exposed to lower salinities at the two temperatures, the O/N ratio ranged from 1 to 10, generally decreasing as salinity decreased (Fig. 6).

Histological responses

In normal seawater (33.0 psu), the external and internal epithelial layers of the mantle of *S. subcrenata* are each composed mainly of a single layer of ciliary conical cells, with the cilia having a well-developed striated border on the free surface (Figs. 7A and 7B).
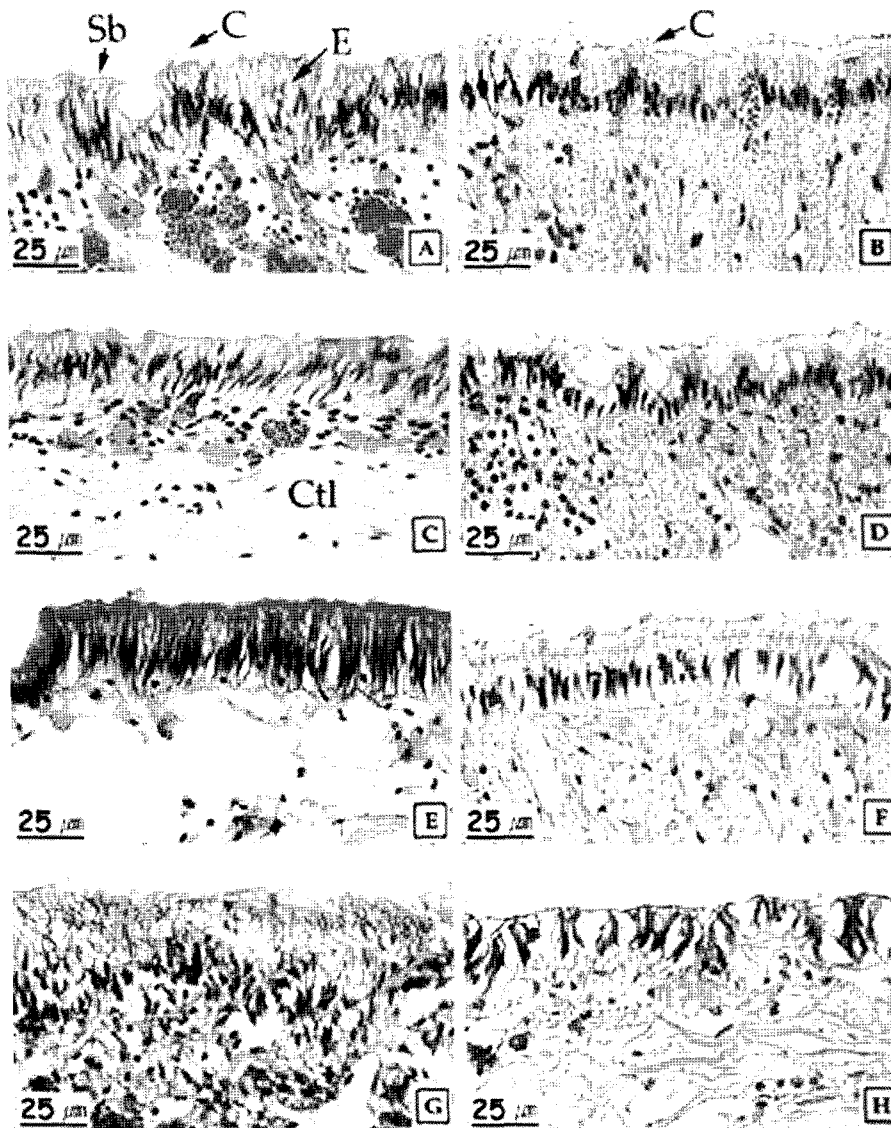
Fig. 7. Histological changes of the mantle of ark shell, *Scapharca subcrenata* exposed to diluted sea water. H-E stain. A, Outer epidermal layer in 33.0 psu at 25°C; B, Inner epidermal layer in 33.0 psu at 25°C; C, Outer epidermal layer exposed to 26.4 psu at 25°C; D, Inner epidermal layer exposed to 26.4 psu at 25°C; E, Outer epidermal layer exposed to 19.8 psu at 25°C; F, Inner epidermal layer exposed to 19.8 psu at 25°C; G, Outer epidermal layer exposed to 13.2 psu at 25°C; H, Inner epidermal layer exposed to 13.2 psu at 25°C; C, cilia; Ctl, connective tissue layer; E, epithelium; Sb, striated border.

When exposed to 26.4 psu, the external epithelial layer dropped the majority of the cilia (Fig. 7C), and the cytoplasm of the epithelial cells of the internal layer enlarged (Fig. 7D). At 19.8 psu, cilia dropped off the external epithelial layer of the mantle, cells became necrotic and vacuolated, and connective tissue was destroyed (Fig. 7E). We also observed necrosis and vacuolation of epithelial cells and destruction of connective tissues in the internal epithelial layer (Fig. 7F). At 13.2 psu, the external epithelial layer and connective tissues of the mantle collapsed

from necrosis (Fig. 7G), while the internal epithelial layer lost most of its cilia, cells vacuolated owing to necrosis, and connective tissue layers were destroyed (Fig. 7H). Thus, the major responses of the mantle of *S. subcrenata* to lower salinity levels included the shedding of cilia, necrosis of epithelial cells, enlargement and vacuolation of cytoplasm, and destruction of connective tissue layers. The degree of these symptoms increased as salinity decreased. In particular, the restoration of mantles exposed to 13.2 psu and 19.8 psu appeared to be impossible.
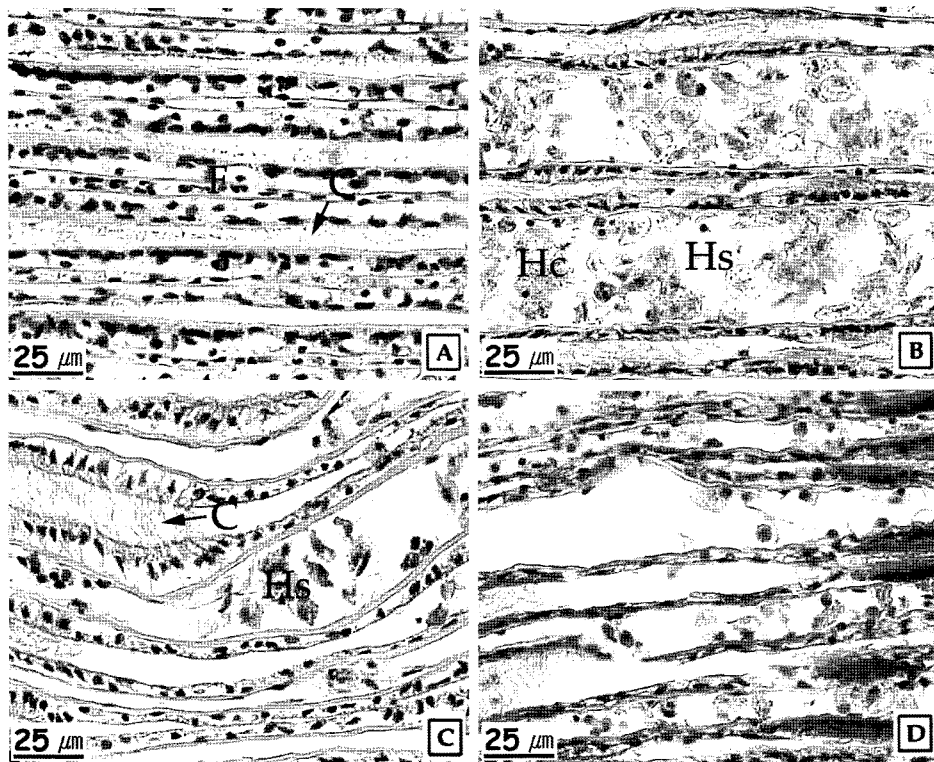
Fig. 8. Histological changes of the gill of ark shell, *Scapharca subcrenata* in diluted sea water. H-E stain. A, Gill exposed to 33.0 psu at 25°C; B, Gill exposed to 26.4 psu at 25°C; C, Gill exposed to 19.8 psu at 25°C; D, Gill exposed to 13.2 psu at 25°C; C, cilia; F, filament; Hc, hemocyte; Hs, hemolymph sinus.

In ordinary seawater (33.0 psu), gill filaments of *S. subcrenata* are arranged in a long row, and cilia are well developed on the free surface of the epithelial layer (Fig. 8A). In gill filaments exposed to 26.4 psu, we observed the contraction of epithelial cell nuclei, partial shedding of cilia, and expansion of hemolymphatic sinuses and increased numbers of hemocytes (Fig. 8B). At 19.8 psu, some filaments displayed more marked shedding of cilia, contraction of epithelial cell nuclei and cytoplasmic enlargement, vacuolation of some epithelial cells, and expanded hemolymphatic sinuses and increased hemocytes (Fig. 8C). Gill filaments exposed to 13.2 psu displayed shedding of cilia, epithelial cell flattening, and epithelial cell necrosis, as well as the exfoliation of part of the epithelial layer (Fig. 8D). Thus, major symptoms of decreased salinity in *S. subcrenata* gills included shedding of cilia, epithelial cell changes, and an increase of hemolymphatic sinuses and hemocytes. The shedding of cilia became more marked at lower salinities, and changes to epithelial cells began to occur, such as nuclear contraction and cytoplasmic enlargement, flattened epithelial cells, and eventual necrosis. The expansion of hemolymphatic sinuses and increase of hemocytes were most prominent at

26.4 psu but decreased as salinity decreased to 13.2 psu.

At 33.0 psu, the visceral mass is composed of several digestive glands and minute vessels. The epithelial layer is composed of single-layered conical epithelial cells and basophiles (Fig. 9A). At 26.4 psu, we observed increased hemocytes, nuclear condensation, and cytoplasmic enlargement, along with some minute particles in the visceral mass (Fig. 9B). At 19.8 psu, we observed nuclear condensation, cytoplasmic enlargement, and the metamorphosis of epithelial cell vacuoles (Fig. 9C). At 13.2 psu, the visceral mass displayed vacuolar metamorphosis due to the necrosis of epithelial cells (Fig. 9D). Minute particles were observed in the digestive glands and minute vessels of the visceral mass at all salinities except 33.0 psu, the salinity of the reference group. Vacuolation became more marked at lower salinities due to epithelial cells necrosis.

Discussion

Temperature and salinity variations are among the most important factors influencing marine organisms (Alderdice, 1972; Ponce-Palafox et al., 1997). Reduced
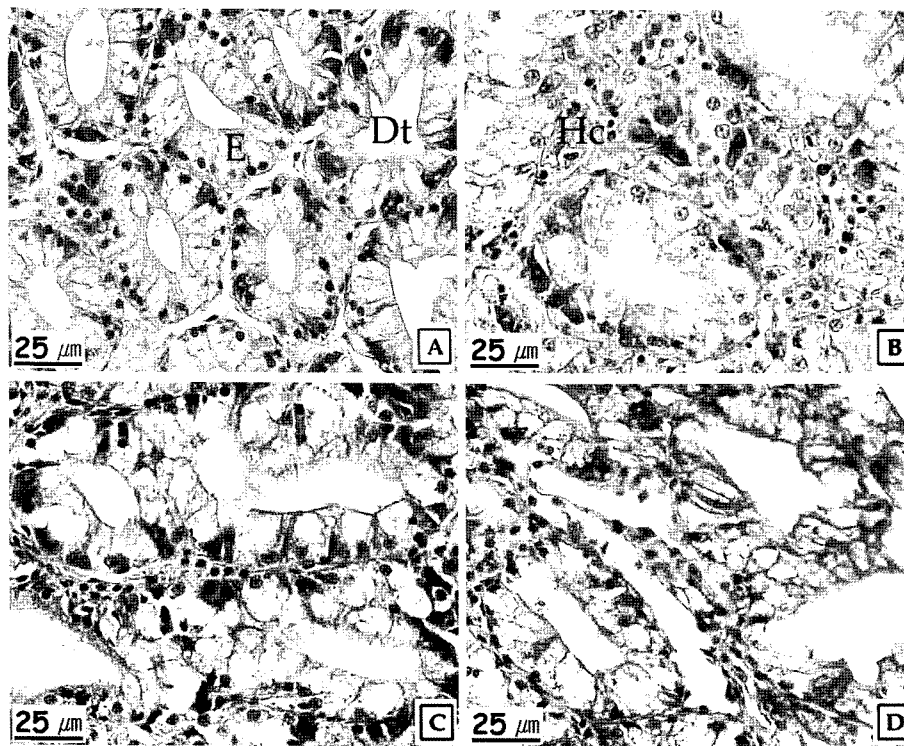
Fig. 9. Histological changes of the digestive gland of ark shell, *Scapharca subcrenata* in diluted sea water. H-E stain. A, Digestive gland exposed to 33.0 psu at 25°C; B, Digestive gland exposed to 26.4 psu at 25°C; C, Digestive gland exposed to 19.8 psu at 25°C; D, Digestive gland exposed to 13.2 psu at 25°C; Dt, digestive tubule; E, epithelium; Hc, hemocyte.

salinity and increased temperature influence metabolic and physiological parameters, including the heart rate of oysters (Feng and Van Winkle, 1975; Bakhmet and Khalaman, 2006), respiration (Shumway and Koehn,1982), and energy acquisition and growth rate (Tolley et al., 2005; Wilson et al., 2005). Although temperature is considered the most important modifier of energy flow and hence of growth, salinity imposes the greatest additional load on the metabolic requirements of aquatic animals. In Korea, *S. subcrenata* aquaculture is concentrated in Yeoja Bay on the southern coast, mainly at depths of 4-6 m. Thus, the bivalve is subjected to substantial climatic influences, including high water temperatures and localized torrential summer rainfalls. Mass mortalities of *S. subcrenata* frequently occur during these summer rainfall events. Hence, we investigated the salinity tolerance of *S. subcrenata* at different temperatures.

Table 1 shows the wide diversity of salinity tolerances of cultured shellfish. The LS_{50} of *S. subcrenata* is 12.59 psu at 25°C and 14 days and 13.87 psu at 15°C and 20 days, thus showing some effect of water temperature. In addition, the LS_{50} at 25°C of *S. broughtonii*, which is cultivated at 10-30 m depth, is

16.5 psu at 9 days, and that of the intertidal zone ark shell *Tegillarca granosa* is 16.8 psu at 11 days, thus highlighting different ranges of salinity tolerance among species of the same family, perhaps stemming from the influence of geographic distribution and habitat (Widdows, 1985) or the unique hereditary background of the organism (Otto, 1973).

Although physiological responses to rapid changes in salinity related to feeding and metabolism are slight at the initial stage of exposure, they adjust as osmotic factors within cells gradually change and recover normal functioning within 2-3 days (Widdows, 1985). The osmolarity of *S. subcrenata* blood when the animal was transferred suddenly from 33.0 psu to 26.4 psu acclimated to the osmolarity of the external concentration within 5 h, and it did so within 20 h when transferred to 19.8 psu. However, when trans-ferred to less than 13.2 psu, the bivalve took more than 60 h to acclimate or died, thus illustrating the limitations of physiological compensation mechanisms that can adjust the energy balance to short-term salinity changes (Widdows, 1985).

A comparison of the lethal critical salinity concentrations of *S. subcrenata* at 15°C and 25°C, which

are 13.87 psu and 12.59 psu, respectively, and their relationship to the osmolarity acclimation of *S. subcrenata* body fluids to external salinity concentrations shows that the time to acclimate to external salinities that approach the lethal critical salinity concentration is prolonged or fatal. Thus, the uniformly balanced state between water content and the influx/efflux of salts within cells appears to be destroyed at concentrations less than the critical concentration (Pierce and Greenberg, 1972), and death results from the energy imbalance caused by the excessive metabolic demand induced by low salinity. The oxygen consumption rate of the experimental groups of *S. subcrenata* was lower than that of the 33.0 psu group throughout the experimental period. Furthermore, although the ammonium excretion rate increased during the initial stage of exposure, it decreased as exposure time lengthened, thereby resulting in changes in oxygen consumption, which is used as a metabolic biomarker of aquatic organisms undergoing stress (Sastry and Vargo, 1977). Our results were similar to the finding that oxygen consumption varies in relation to environmental changes (Almada-Villela, 1984). In addition, although the oxygen consumption rate, clearance rate, and energy balance were uniformly maintained at 20.3 psu in *Mytilus edulis*, metabolism was reduced at less than 20.2 psu (Widdows, 1985), suggesting that, although metabolic adjustment is possible in response to salinity changes within the physiological range, the ability to adjust deteriorates as metabolism decreases in conditions outside the physiological range, thereby resulting in death.

The ratio between oxygen consumed and nitrogen excreted in atomic equivalents (O/N ratio) represents the degree to which protein is utilized in energy metabolism by marine invertebrates (Shumway and Newell, 1984). According to Bayne (1973), O/N values above 50 are representative of healthy mussels, whereas values of 30 or below generally indicate stressed animals with relatively high protein catabolism. Mayzaud (1973) found that the minimum O/N ratio value is 7, which indicates exclusively protein catabolism. The O/N ratio of *S. subcrenata* varied between 27 and 1, with values decreasing with decreasing salinities. At 15°C and 25°C, O/N ratios in all groups except the control group (33.0 psu) were below 10, indicating that ark shells are influenced by salinity reductions. Especially at 25°C, O/N ratio values were below 5, indicating that ark shells expend more protein as energy to acclimate to lower salinity (Mayzaud, 1973). The effects of low salinity on *S. subcrenata* can be observed in the histological

changes of organs. As salinity decreased, we observed shedding of cilia, necrosis and vacuolation of the epithelial cell layer, and destruction of connective tissue layers in the mantle and visceral mass, as well as necrosis of the epithelial cell layer and increase of hemocytes in the gills. The main phenomenon of decreased salinity is cell necrosis, which leads to increased salt imbalance due to osmotic concentration differences, eventually resulting in death.

Yeosu Bay, one of the largest *S. subcrenata* aquaculture locations in Korea, has been under culture for more than 20 years. However, mass mortalities of *S. subcrenata* have sometimes occurred, and currently the yield is dropping. The cause of this decline is unclear, and physiological data on *S. subcrenata* do not provide sufficient information. Thus, the appropriate range of environmental tolerance of *S. subcrenata* must be examined to manage *S. subcrenata* aquaculture sustainably and preserve an important source of aquacultured shellfish.

Acknowledgements

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