The osmoregulatory ability and salinity tolerance of four species of decapod crustaceans (Decapoda: Natantia) were evaluated to test the degree of physiological adaptation to variations in salinity (2 d exposure to 10, 20, 25 and 32). The ecophysiological salinity tolerance of two penaeids (Metapenaeus joyneri and Penaeus semisulcatus) and two palaemonid species (Palaemon pacificus and Exopalaemon orientalis), representative of natant crustacean species inhabiting coastal shallow-water environments were evaluated. Our results show that both E. orientalis and P. pacificus are tolerant of all salinities tested here due to displaying both hyper-/hypo-osmoregulatory ability (in low and high saline waters, respectively), consistent with osmoregulatory patterns displayed by other species in the family (Palaemonidae). The two penaeid species evaluated here displayed efficient hyporegulatory ability in full-strength seawater; however, high mortality was observed in both species at low salinity exposure (10) (73% and 87% mortality for M. joyneri and P. semisulcatus, respectively). In terms of salinity tolerance and thus osmoregulatory ability (which is the mechanism of euryhalinity) of the four species, the following relative order could be assumed: Palaemon pacificus > Exopalaemon orientalis > Metapenaeus joyneri > Penaeus semisulcatus.

Key words: Crustacea, East China Sea, osmoregulation, physiology, salinity

Introduction

Physiological responses have long been used as methods to determine how decapod crustaceans respond to changes in their environment (e.g. abiotic factors, such as temperature and salinity), i.e. ecophysiology (Spicer & Gaston 1999). Physiological parameters, and thus indicators of ecophysiological performance, include temperature and salinity tolerance (Chen & Lai 1993), oxygen consumption rates as surrogates for metabolism (Dalla Via 1986, Walker et al. 2009) and growth and survival (Chen et al. 1992, Cha et al. 2004, Kumlu & Kir 2005). Tolerance to abiotic conditions generally defines species geographic boundaries, e.g. at small spatial scales, such as vertical distribution along rocky shores (Little & Kitching 1996) or at large spatial scales, such as latitudinal gradients (Tewksbury et al. 2008). However, even if tolerance to certain conditions may be exhibited, it does not necessarily equate to the optimal organism ecophysiological performance (Bray et al. 1994), especially in terms of anthropogenic climate change (i.e. increased temperatures and ocean acidification), and includes changes to coastal water environments, such as variable precipitation events, increased run-off, saltwater intrusion into inland waters and increased temperature and 'ocean acidification' (increased seawater acidity) (FAO 2008a, Hofmann & Todgham 2010). Tropical species are thought to be close to thermal and thus physiological limits (Tewksbury et al. 2008), which is hypothesised to impose further strains on organism physiological limits (Stillman 2003, Pörtner & Farrell 2008).

The physiological ability to tolerate wide salinity fluctuations or euryhaline tolerance is due to osmoregulatory ability. Osmoregulation is the regulation of the total particle concentration of body fluids at levels different from those of the external medium (Robertson 1960). Thus, hyper- and hypo-osmoregulators maintain levels above or below that of the external medium in low saline and fully-saline waters, respectively. With regard to the Natantia decapods, specifically, the penaeids and palaemonids display efficient os-
moregulatory abilities, thus allowing these species to inhabit both freshwater and marine biotopes (Freire et al. 2003).

The aim of this study was to evaluate the osmoregulatory (hyper/hypo-osmoregulatory) ability and salinity tolerance of four decapod crustacean species (Decapoda: Natantia), thereby elucidating the degree of physiological adaptation to freshwater and full-strength seawater. The present study evaluates four ecologically-distinct species: two palaemonids (Palaemon pacificus Stimpson and Exopalaemon orientalis Holthuis) and two penaeids (Metapenaeus joyneri Miers and Penaeus semisulcatus De Haan). These four species were chosen as representative natant decapods occupying various coastal shallow-water habitats, as well as for their economic importance as commercial fishery species in and around the East China Sea basin (Holthuis 1980, Cha et al. 2004) with exception of P. pacificus. Palaemonids, however, mainly inhabit brackish-water to marine habitats and are efficient hypo-regulators in seawater. Penaeids provide high nutritional value as seafood and contribute ca. 20% of the average per capita protein intake for over 2.8 billion people, mostly from developing countries from the subtropical and tropical latitudes (FAO 2008b). Animals from freshwater, brackish-water and marine contribute 57%, 7.4% and 36%, respectively from aquaculture (FAO 2005), thereby underscoring the importance of this crustacean family. Species from these two families (Peneidea and Palaemonidae) are fished commercially as they are moderate to large in size (5–15 cm) and often occur in large quantities in shallow waters and along the continental shelf (Chan 1998); palaemonids, however, comprise 26% of the global shrimp catch (919 088 tonnes) (FAO 2008b). The implications of this research may allow for the assessment of physiological tolerances to changing salinity which may occur due to climate change, where changes in precipitation and evaporation may occur in waters from tropical latitudes (IPCC 2007).

**Materials and Methods**

All individuals were collected from a series of habitats within the East China Sea, Japan ranging from marine tide pools through estuaries during July 2009 [ambient mean temperature 22±1°C, as found previously (Minagawa et al. 2000)]. *Palaemon pacificus*, a marine species was collected from tidal pools using hand-held nets at the point of low water spring (LWS) from Teguma Harbour, Nagasaki, Japan (32°46'45"N, W 129°47'44"W). *Exopalaemon orientalis* and *Metapenaeus joyneri* were obtained from local fishermen in Ariake Bay, Saga, Japan. Juvenile *Penaeus semisulcatus* were donated by Saga Prefectural Genkai Fisheries Research and Development Center, East China Sea, Japan. Each was measured [body length (BL) mm: rostrum to telson; see Table 1] and all species were held separately, and maintained in holding tanks (vol. 250 L) containing filtered (10 μm carbon-filtered) continuously-circulating aerated seawater 22±1°C, pH 8.15±0.05; under a 12 h light: 12 h dark photoperiod. Individuals were held for a maximum for a week and fed commercial feed pellets every two days (ca. 3% body mass).

Animals were acclimated in salinities as recorded from each habitat at time of collection (Table 1) (salinity of 35 for all species, except salinity of 18 for *Exopalaemon orientalis*) prior to salinity tolerance exposure (one-step dilution) to salinities of either 10, 20, 25 or 32, chosen to represent ‘freshwater’ (hyper-osmotic) (10), isosmotic (20–25) or full strength seawater (hyper-osmotic) (32) salinities. For salinity tolerance experiments, animals were held individually for 48 h in 2.3 L tanks; 2 day salinity acclimation periods were chosen as crustaceans can generally tolerate sudden changes in salinity as the osmoregulating enzyme (Na+/K+ ATPase) usually up-regulates within 48–72 h (reviewed in Whiteley et al. 2001) and haemolymph osmolality stabilising within 12–48 h (Tantulo & Fotedar 2006). Seawater (salinity of 35) was diluted to attain the desired test salinities (32, 25, 20 or 10) using de-ionised freshwater (0 μS cm⁻¹) which had been filtered through an ion-exchange column (GC-10c, Organo, Japan). Media salinity was determined using a conductivity/salinity meter (SevenGo, Mettler Toledo). All salinity exposures were conducted at 22±1°C.

Haemolymph (25 μL) was extracted using an ice-chilled Hamilton microsyringe. After drying the cephalothorax using absorbent paper, the needle was inserted into the pericardial cavity between the thorax and first abdominal segment (Campbell & Jones 1989). All measurements were taken within 10 s of haemolymph sampling from each individual in order to prevent clotting. Haemolymph osmolality (mosmol kg⁻¹) was determined using a vapour pressure osmometer (Wescor 5520, USA). Results were expressed either as haemolymph osmolality or as osmoregulatory capacity (OC), defined as the difference between the osmolalities of haemolymph and the medium; positive and negative values indicate hyper- and hypo-osmoregulatory capacities, respectively (Salinity of 1=29.41 mosmol kg⁻¹) (Charmantier & Anger 1999). Haemolymph osmolality represents the actual value internally and OC represents the capacity of the animal in relation to the external environment i.e. water. Isosmotic points were calculated using linear regressions of the OC linear curve and expressed as isosmotic salinities (Equation 1a) and haemolymph osmolalities (Equation 1b) (see equation below); linear regression values are summarised in Table 1.

**Equation 1.**

a. Isosmotic salinity=*(haemolymph osmolality)/slope*
b. Isosmotic point=*(isosmotic salinity *29.41 mosmol kg⁻¹)*

Analysis of Variance (ANOVA) tests were performed to test for differences [as revealed by the post-hoc Student–
Results

All species maintained the internal osmolality (haemolymph) above that of the media (water) at the hypo-osmotic salinity (salinity of 10) and internal osmolality below medium salinity at the hyper-osmotic salinity (salinity of 32), except for *Penaeus semisulcatus* (Fig 1A–D). Osmotic capacity evaluation of all species demonstrated a negative linear relationship of osmotic capability with increasing salinity (Table 1), thus demonstrating the osmoregulatory ability of all species.

Both palaemonids (*Exopalaemon orientalis* and *Palaemon pacificus*) hyper-regulated in salinity of 10, where haemolymph osmolality was higher than that of the media (Fig. 1A–B). Haemolymph osmolality (mean±SE) was highest in *P. pacificus* (588±22), indicating that they were the highest hyper-regulators. Comparatively, *E. orientalis* (499±15) had the lowest haemolymph osmolality, thereby indicating that they were significantly relatively weaker hyper-regulators (*F*3,36=12.44, *p*<0.001) (Fig. 1B). Significant mortality was observed in the penaeids, *Metapenaeus joyneri* and *Peneaus semisulcatus* (73% and 87%, respectively) (Table 1). All species hypo-regulated in seawater (salinity of 32); haemolymph osmolalities were significantly higher in the palaemonids than the penaeids: *Exopalaemon orientalis* (549±6) and *P. pacificus* (645±13) vs. *M. joyneri* and *P. semisulcatus* (478±17 and 463±13, respectively) (Fig. 1).

Osmotic capability plotted against salinity allowed for calculation of the isosmotic points of all species (isosmotic osmolality and salinity) (summarised in Table 1). Three species (*P. pacificus, E. orientalis* and *M. joyneri*) displayed similar (calculated) isosmotic points (mean haemolymph osmolality ca. 650 and salinity of 22). In general, the palaemonids were more tolerant of low salinity exposure than the penaeids, as observed by no mortality at 10 and efficient hyper-regulation. *Metapenaeus joyneri*, although it has a similar isosmotic point compared with both palaemonids, generally could not hyper-regulate well in freshwater (salinity of 10), as observed by the high mortality (73%). The isosmotic point for *Peneaus semisulcatus*, however, was calculated as 14 and could not generally tolerate the lower salinities tested here, as high mortalities were observed (87 and 43% mortality at salinities of 10 and 20).

**Discussion**

In general, all species displayed either or both hyporegulation in ‘freshwater’ and hyper-regulation in seawater. *Palaemon pacificus* and *Exopalaemon orientalis*, even

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### Table 1. Physiological summary data (mean±SE) for several decapod (Natantia) species. HO signifies mean haemolymph osmolality (mosmol kg\(^{-1}\)), OC signifies mean osmotic capacity.

<table>
<thead>
<tr>
<th>Species</th>
<th>Palaemon pacificus (Stimpson)</th>
<th>Exopalaemon orientalis (Holthuis)</th>
<th>Metapenaeus joyneri (Miers)</th>
<th>Peneaus semisulcatus (De Haan)</th>
</tr>
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<tbody>
<tr>
<td>Body length (mm) (mean±SD)</td>
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</tr>
<tr>
<td>Common name (Japanese)</td>
<td>Isoshiji ebi</td>
<td>Shirata ebi</td>
<td>Shiba ebi</td>
<td>Kuma ebi</td>
</tr>
<tr>
<td>Common name (English)</td>
<td>Indian bait prawn</td>
<td>Oriental prawn</td>
<td>Shiba shrimp</td>
<td>Green tiger prawn</td>
</tr>
<tr>
<td>Habitat</td>
<td>East China Sea rocky shore</td>
<td>Ariake Bay (Brackish water)</td>
<td>Ariake Bay (Brackish water/Marine)</td>
<td>East China Sea</td>
</tr>
<tr>
<td>Salinity at habitat</td>
<td>35</td>
<td>18</td>
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<td>35</td>
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<tr>
<td>Exposure media</td>
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<td>salinity</td>
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<td>Osmotic capacity linear</td>
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<td>regression equations (r(^2))</td>
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<td>Osmotic point (calculated)</td>
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<td>Isosmotic salinity (calculated)</td>
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</table>
though they are adapted to differing saline regimes (fully-marine and estuarine respectively; Table 1), both display efficient hyper-/hypo-osmoregulatory abilities, as displayed by other palaemonids [e.g. *P. elegans* Rathke (Ramirez de Isla Hernandez & Taylor 1985); *P. serratus* Pennant (Panikkar 1940, Parry 1957); *P. affinis* Milne Edwards (Kirkpatrick & Jones 1985)] and *P. northropi* Rankin (Freire et al. 2003]). The osmoregulatory ability and salinity tolerance pattern observed here indicates that, although both palaemonid species (*E. orientalis* and *P. pacificus*) are acclimated to varying salinity regimes, the mechanism for euryhalinity remains conserved (i.e. hyper-regulating in freshwater and hypo-regulating in seawater).

With respect to isosmotic points, three species (*P. pacificus* (n=13 per salinity); *B: Exopalaemon orientalis* (n=10 per salinity); *C: Metapenaeus joyneri* (n=15 per salinity); *D: Penaeus semisulcatus* (n=15 per salinity). X axis is denoted both in terms of salinity and osmolality (mosmol kg\(^{-1}\)). Solid lines denote isosmotic relationship where internal osmolality matches that of the exposure media; polynomial regression curves are fitted (dotted line) to denote osmoregulatory ability (\(r^2=0.96–1\)).
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cus, E. orientalis and M. joyneri) display isosmotic salini-
ties (salinity of 22) similar to other palaemonid species [P
elegans (salinity of 24) and P. serratus (salinity of 25)
(Panikkar 1940, 1941)] and other penaeids (reviewed in Se-
tiarto et al. 2004). Metapenaeus joyneri and Penaeus semi-
sulcatus individuals from either species could not tolerate
the lowest salinity tested here (salinity of 10) as observed
by high mortalities (73% and 87%, respectively). The in-
ability of both adult M. joyneri and juvenile P. semisulcatus
to tolerate freshwater salinity could be explained by the in-
ability to hyper-regulate in lower salinities, as shown in an-
other penaeids (reviewed in Mantel & Farmer 1983, Minh
Sang & Fotedar 2004). The poor salinity tolerance in low
salinity and hypo-osmoregulatory pattern in seawater ob-
served here in juvenile P. semisulcatus matches the pattern
observed in adults, except a higher mortality rate is ob-
served in freshwater (100%) (Clark 1992). Contrastingly, P.
semisulcatus displays the converse pattern to another pe-
naeid species Marsupenaeus japonicus, which hyper-regu-
lates in seawater (Charmantier-Daures et al. 1988, Setiarto
et al. 2004). With regard to M. joyneri, other congeneric
species display efficient osmoregulatory abilities at both
low and high saline waters, respectively, e.g. Metapenaeus
bennettae (Dall 1981), thus suggesting a dichotomy in os-
moregulation patterns even within the same genus of deca-
pod crustaceans.

In terms of euryhalinity, as determined both by the os-
moregulatory ability and salinity tolerance, the following
relative order of best to worst could be assumed: Palaeon
pacificus > Exopalaemon orientalis > Metapenaeus joyneri >
Penaeus semisulcatus. Penaeid shrimp, in general as juve-
niles, may be physiologically adapted to life in coastal areas,
estuaries and lagoons, where medium salinity is typi-
cally variable, but lose this capacity as they grow and gradu-
ally migrate into deeper waters (Setiarto et al. 2004). Juve-
nile Penaeus semisulcatus, as shown previously (Kumlu
et al. 2000) and described here, indicate that the osmoregula-
tory ability and salinity tolerance (to freshwater) is similar
to the adult form and may be at risk in seawater dilution
events (e.g. precipitation) as well as variable salinity
regimes in estuarine coastal waters (Vance 1996).

The regulatory abilities assessed here reflects the effi-
cient hyper-/hypo-osmoregulatory capabilities of two pala-
emonids compared with two penaeids and show that pe-
naeids may be comparatively intolerant of wide salinity
fluctuations. Climate change is purported to impact broadly
on the biota in coastal ecosystems, fishery communities,
aquaculture industries and the human population dependent
upon them. As penaeids are an important coastal economic
resource in subtropical/tropical latitudes, it is imperative
that further physiological evidence is gathered in order to
identify the physiological limits and adaptability potential
of this crustacean family to variable environmental factors.
Calculation of the osmoregulatory capacity (OC) could be
used as an effect tool in monitoring individual physiological
condition and the effect of stressors (Minh Sang & Fotedar
2004). Thus, in order to evaluate the adaptability of species
to abiotic factors and changing ecosystems, we advocate
the use of ecophysiological studies in key model groups
with regard to single (as observed here) and synergistic fac-
tors, such as temperature, salinity, and ocean acidification,
i.e. future climate scenarios.

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