國立臺灣師範大學生命科學系碩士論文

三種闊尾海蛇滲透壓調節能力之比較研究

Comparison of the osmoregulatory capability among three sea snakes (*Laticauda* spp.)

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摘要

滲透壓與離子的調節與恆定是海洋脊椎動物面臨的生存挑戰之一，而個體的滲透壓調節能力往往與其海棲程度有相關性。海蛇的後端舌下腺(posterior sublingual gland)為其鹽腺，能夠排出濃縮的氯化鈉以維持滲透壓的恆定。鹽腺管狀構造之上皮由主細胞(principal cell)構成。主細胞底側膜上 Na^+/K^+ -ATPase (NKA)以耗能的主動運輸將體內過多的鹽分排除體外。三種不同海棲程度的闊尾海蛇(*Laticauda* spp.)分布於台灣的蘭嶼及綠島；闊帶青斑海蛇(*L. semifasciata*)是海棲程度最高的物種，黑唇青斑海蛇(*L. laticaudata*)次之，而黃唇青斑海(*L. colubrina*)蛇是最陸棲的物種。本實驗目的為比較台灣三種闊尾海蛇的滲透壓調節力是否有所差異並呼應其海棲程度。將三種闊尾海蛇分別從陸域轉移至海水及淡水馴養，分析轉移後的第 0, 2, 7, 14 天的血漿滲透壓、血漿離子濃度、血球容積比、肌肉含水量以及鹽腺和腎臟的 NKA 活性變化，用這些指標來分析其滲透壓調節能力。結果顯示，相較於黑唇及黃唇青斑海蛇，海棲程度最高的闊帶青斑海蛇維持體液滲透壓、鈉氯離子濃度以及肌肉含水量最為穩定。闊帶青斑海蛇及黑唇青斑海蛇的鹽腺 NKA 活性在海水馴養組皆顯著高於淡水馴養組，而陸棲程度最高的黃唇青斑海蛇的鹽腺 NKA 活性不論轉移至海水或

淡水都沒有顯著變化。以上證據顯示闊帶青斑海蛇的滲透壓調節能力較黑唇青斑海蛇及黃唇青斑海蛇佳。而最陸棲的黃唇青斑海蛇似乎採取不同於闊帶與黑唇青斑海蛇的滲透壓調節策略因應環境鹽度變化。

關鍵字：闊尾海蛇、鹽腺、滲透壓調節、鈉鉀幫浦

Abstract

Marine invasions have occurred multiple times independently among vertebrates. To permit the successful habitation of marine environments, the specialized ionoregulatory tissues have evolved, likely been responsible for ameliorating ionic challenge. Therefore, salt gland has evolved multiple times throughout the evolution of marine vertebrates. The sublingual salt gland is the primary organ of salt excretion in sea snakes, and Na^+/K^+–ATPase (NKA) in the basolateral membrane provides the driving force for salt secretion. In this study, the osmoregulatory capability of three sea kraits (*Laticauda* spp.) in Taiwan were examined and compared to test if their osmoregulatory capability is associated with their different habitats affinity from terrestrial to marine. The sea kraits were transferred from terrestrial environment to freshwater (FW) or seawater (SW) for 0, 2, 7, and 14 days. At various time points, their salt glands and kidneys were sampled for NKA activity analysis; muscles were sampled for water content measurement; blood were sampled for the analysis of hematocrit, osmolality, and ionic concentrations. Results showed that the most marine species, *L. semifasciata* maintained better constancy in plasma osmolality, Na^+, Cl^- levels and water content. In *L. semifasciata* and *L. laticaudata*, the NKA activity of the salt gland was higher in SW than in FW. However, in the most terrestrial species, *L. colubrina*, no significant difference of NKA activity was found between SW and FW groups. These results suggest that the capability of osmoregulation is better in

L. semifasciata than in the other two species, and *L. colubrina* may have different osmoregulatory strategy with the other two species.

Keywords: sea krait, salt gland, osmoregulation, Na^+ /K^+ -ATPase

Introduction

Osmoregulation in marine non-mammalian vertebrates

There are numerous animal groups originating in freshwater and terrestrial habitats, which have recolonized the marine environment called secondary marine organism (Vermeij and Dudley, 2000; Willmer et al., 2004). Among vertebrates, marine invasions have occurred multiple times independently. When marine invasion occurred, salinity poses a strong physiological barrier to terrestrial vertebrates. The salinity of seawater is appropriately 1000 mOsm Kg^{-1}, however, most terrestrial vertebrates maintain their body fluid appropriately between 250~450 mOsm Kg^{-1}. In marine environment, vertebrates experience dehydration or salt accumulation because of hyperosmotic solution (Willmer et al., 2004). For a successful habitation, varieties of osmoregulatory tissues and organs have been evolved among marine vertebrates, which are likely to response to this osmotic challenge. Gills are the osmoregulatory tissue of teleosts (Evans et al., 2005), and salt glands are the tissues excreted salt in marine birds, reptiles and elasmobranches (Burger, 1965; Schmidt-Nielsen, 1958; Schmidt-Nielsen, 1960).

In marine teleosts, the net osmotic loss of water and diffusional gain of salt across the gills is balanced by ingestion of sea water, production of small volumes of urine that contains some salt (to minimize urine loss of water), and active excess salt *via* the gill (Evans, 2009a). Marine elasmobranches have a different strategy for osmoregulation, they retain

urea and trimethylamine oxide (TMAO) in blood to avoid the desiccating effect of sea water, because these two osmolytes raise the osmolality of their blood slightly higher than that of sea water. The gain of water and salt across the gill is balanced by production of a large volume of urine that contains some salt, active secretion of salt by the rectal gland (Anderson et al., 2007; Hammerschlag, 2006; Evans 2009a). Similar to marine teleosts, marine birds maintain water balance by ingestion of sea water, and then orbital salt gland secretes excess salt (Evans, 2009c; Hughes, 2003).

In reptiles, several kinds of salt gland have been investigated: lachrymal glands in sea turtles and terrapins (Dunson, 1970; Hudson and Lutz, 1986), nasal glands in marine iguanas (Dunson, 1969), lingual glands in crocodilians (Taplin and Grigg, 1981), posterior sublingual glands (PSG) and pre-maxillary glands in marine snakes (Dunson and Dunson, 1973; 1979; Dunson et al, 1971). Although current opinions believe that marine reptiles can exist in marine environment without fresh water is because of the ability of desalinate salt water by their salt glands (Heatwole, 1999; Randall, et al., 2002), many cases were reported that marine reptiles drink fresh water in the field (Bonnet & Brischoux, 2008; Taplin, 1984) or laboratory (Dunson and Robinson, 1976), or need to maintain water balance through the ingestion of fresh water (Lillywhite and Ellis, 1994; Lillywhite et al., 2008). In addition, marine reptiles minimize dehydration in seawater by the corneum of their thick skin (Vitt and Caldwell, 2009; Evans, 2009b). Because of absence of the loop of Henle in kidneys, they cannot excrete highly concentrated urine;

alternatively, salt gland is the primarily site of salt excretion (Evans, 2009b).

Structure of Salt glands

Salt glands are usually comprised by branch of secretory tubules which are winding around a central duct and forming a lobe by the bindings of connective tissues. Multiple lobes are joined by central ducts to the main duct, thus the secretory fluids can transfer from the tubules to the expelled site (Chan and Phillips, 1967; Franklin et al., 2005; Kent and Olsen, 1982; Peaker, 1971). Principal cells and peripheral cells are two typical cells in tubules of salt glands. Principle cells have dense mitochondria and either deeply invaginated basal membranes (birds) or extensive lateral evaginations (elasmobranchs) which provide the surface area necessary to house the suite of membrane-bound, ion transport proteins that typify vertebrate secretory cells (Kirschner, 1980; Lowy et al., 1989; Ernst et al., 1994; Riordan et al., 1994). Principal cells are most abundant in the tubular epithelia and mediate the salt secretory function (Shuttleworth and Hildebrandt, 1999).

Mechanism of NaCl secretion in salt glands

The physiological mechanisms of salt secretion have been studied explicitly in the gills of seawater teleosts (Evans et al., 2005; Hwang and Lee, 2007; Marshall, 2002) and in the salt glands of marine birds and

elasmobranches (Hildebrandt, 2001; Riordan et al., 1994; Shuttleworth and Hildebrandt, 1999; Silva et al., 1997), however, little is known about marine reptiles' salt glands.

In the gills of marine teleost, three ion transport proteins have been found to play a critical role in salt secretion of the functional cells, mtochondrion-rich (MR) cells (Marshall, 2002). They are Na^+/K^+ -ATPase (NKA), $Na^+/K^+/2\ Cl^-$ cotransporter (NKCC) and cystic fibrosis transmembrane conductance regulator (CFTR). The basolateral NKA pump Na^+ out of the MR cells and the accumulated Na^+ is extruded by a transepithelial electrochemical gradient. The Na^+ gradient between the basolateral membrane of MR cells drives NKCC to carry Na^+, K^+ and 2 Cl^- into the MR cells. Then the accumulated Cl^- in MR cells diffuse out of the cells *via* a Cl^- channel (CFTR) on the apical membrane. The NKA in MR cells is thought to play a dominant role because it provides the primary driving force for Na^+ and Cl^- secretion. Therefore, the NKA activity in fish gills has been used as a parameter to monitor the capability or demand of salt secretion (Hwang and Lee, 2007). In the salt glands of marine birds and elasmobranches, the three ion transport proteins (basolateral NKA, NKCC and apical Cl^- channel) were observed in the secretory principal cell (Hildebrandt, 2001). Basolateral NKA, NKCC and apical Cl^- channel are involved salt secretion, and the mechanism is similar to marine teleost (Hildebrandt, 2001). Only a few studies focus on the structure and function of salt gland of reptiles in recent decades (Cramp et al., 2008; Reina and Cooper, 2000; Reina et al., 2002; Babonis et al., 2009; Cramp et al., 2010).

Role of Kidney in osmoregulation

Due to lacking of Henle's loop, marine teleost, elasmobranchs and reptiles cannot produce highly concentrated urine (Evans, 2009a; b; c). Therefore, kidneys do not contribute much to the salt secretion in these marine vertebrates. In euryhaline teleosts and elasmobranchs, the renal NKA activity was found to be higher in FW individual than in SW individual (Lin et al., 2004b; Madsen et al., 1994; Pillans et al., 2005; Venturini et al., 1992). The high NKA activity in kidney of FW acclimated fishes is considered as a upregulation of ion reabsorption since FW fishes have to excrete a large amount of diluted urine (Perry et al., 2003). In marine reptiles, the kidney is also thought to play an important role in water reabsorption but not in salt excretion (Benyajati et al., 1985; Grigg, 1981; Kuchel and Franklin, 1990; Yokota et al., 1985).

Osmoregulation in sea snakes

The posterior sublingual gland (PSG) of sea snakes secreted concentrated salt solution after a salt loading, suggesting that it is the salt gland of sea snakes (Dunson et al, 1971). The PSG lies on the ventrolateral surfaces of the tongue sheath and its morphology is similar to the other salt glands in marine birds and elasmobranches (Babonis et al., 2009). The principal cells are the most abundant cells on the tubular epithelium of PSG. The PSG is comprised by several branches of

secretory tubules, which are bounded by connective tissues. All of the tubules are joined to a central duct, which is connected to the tongue sheath (Babonis et al., 2009). Like marine elasmobranchs and birds, the principal cells of PSG exhibiting the lateral evaginations typical of elasmobranch principal cells (Dunson and Dunson, 1973; 1979). The NKA activity was detected in salt glands from various taxa of sea snakes (Dunson and Dunson, 1974; Dunson and Dunson, 1975), and NKA and NKCC were immune-localized to the basolateral membrane of principal cells in three species of sea snakes (Babonis et al., 2009). It suggested that the mechanism of NaCl secretion in sea snake is similar to the salt glands in other marine vertebrates.

Like other marine reptiles, sea snakes' cuticle consists of thick skin, which can minimize dehydration in hypertonic sea water. Early studies reported that the skin of sea snakes is permeable to water but not Na^+ (Dunson and Robinson, 1976; Dunson, 1978), suggesting that sea snakes experience dehydration rather than salt accumulation when inhabit in marine environment. Some evidence suggests marine snake cannot prevent dehydration in SW (Lillywhite and Ellis, 1994; Lillywhite et al., 2008).

Three sea kraits in Taiwan

Numerous groups of snakes were found to inhabit in marine environment. Four taxa (family or subfamily) of snakes contain marine

species, including Hydrophiidae (true sea snakes), Laticaudinae (sea karits), Colubridae (Colubrids) and Acrochordidae (file snakes) (Heatwole, 1999). Some species in Laticaudinae and Colubridae still retain connection to terrestrial environments; they rest, shed, mate, lay eggs on land. Although Acrochordidae is entirely aquatic, they have to go to freshwater environment and cannot always stay in seawater. Only Hydrophiidae snakes are fully marine species (Heatwole, 1999). However, the "sea snake" usually indicates the two groups of snakes, Hydrophiidae and Laticaudinae. They are venomous elapid snakes and inhabit marine environments for most or all of their life. There are some taxonomic arrangements among sea snake, however, it seems clear that all of the lineages that are referred to as "sea snakes" are closely related and originated from terrestrial elapid ancestors, the Laticaudinae and the Hydrophiidae do not form a monophyletic group and likely represent two separate invasions of the marine environment (Keogh 1998). The Laticaudinae only has one genus: *Laticauda* and eight species (Cogger and Heatwole, 2006).

There are three species of *Laticauda* distributed in Orchid Island of Taiwan; they are *Laticauda semifasciata*, *L. laticaudata* and *L. colubrina* (Tu, 2004). Two species (*L. laticaudata* and *L. colubrina*) are distributed in Green Island of Taiwan (Tu, 2004). The three species were reported to have different habitat affinities (Liu and Tu, unpublished data). *L. semifasciata* is the most marine species, they seldom come ashore. On the contrary, *L. colubrina* is the most terrestrial species; they spend considerable time ashore and spending

half their time out of sea and moving considerably inland (Shetty and Shine, 2002). The third species, *L. laticaudata* is the species in between; they spend relatively short time ashore and usually rest in the narrow intertidal zone (Liu and Tu, unpublished data). A previous study measured the dehydration rate of the three species out of water and found no difference between *L. semifasciata* and *L. laticaudata*, whereas *L. colubrina* was lower than the other two species (Lillywhite et al., 2008). The dehydrated sea kraits were reported to drink diluted sea water but not full strength sea water (Lillywhite et al., 2008). The salinities of the water they choose to drink are also different among the three species; *L. semifasciata* drunk 30% SW, *L. laticaudata* preferred 20% SW and *L. colubrina* drank the most diluted SW (10%) (Lillywhite et al., 2008). Moreover, the skin permeability to water is also different among them; *L. semifasciata* was the highest, *L. colubrina* was the lowest, and *L. laticaudata* was the species in between (Lillywhite et al., 2009).

Objective

Osmoregulation is important for marine organisms, and the capability of osmoregulation is associated with organisms' habitat affinity (Bennett and Hughes, 2003; Hiroi and McCormick, 2007; Rigal et al., 2008; Taplin et al., 1982). About the salinity effect on marine reptiles' habitat, Dunson and Mazzotti (1989) suggested that salinity as a limiting

factor in the distribution of reptiles in Florida bay. It has been shown the three sea kraits in Taiwan perform different habitat affinities and they also have different resistance to water loss (Lillywhite et al., 2009). However, their capabilities or strategies responding to osmotic challenges have not been fully investigated. In this study, I attempted to compare the osmoregulatory parameters among the three Taiwan sea kraits and tried to study if there is a correlation between their physiological properties and ecological properties. The NKA activity of salt gland, plasma osmolality, ion concentrations, hematocrits of blood, and muscular water content were analyzed in the three species subjected to osmotic challenges.

Materials and Methods

Animal collections and experimental protocols

Three species of adult male sea kraits were collected mostly from Orchid Island of Taiwan, and a few *L. laticaudata* and *L. colubrina* were collected from Green Island of Taiwan. The field work of collection was during August to September of 2009 and April to August of 2010. Animals were captured by hand from the shallow coastal inlets and were transported to the laboratory of National Taiwan Normal University in Taipei within 7 days. The animals were kept in plastic tanks (36.5 × 23.3 × 15.6 cm^3) and provided dechlorinated tap water for drinking or soaking for one week. Then, 5 to 8 individuals of each species were initially sampled (presented as FW/ SW0), and the remainders were randomly transferred to fresh water (FW, dechlorinated tap water) or 32 ppt sea water (SW, prepared by artificial sea salt, Instant Ocean™ powder, Crystal Sea, Baltimore, MA, USA). The animals were confined in the water. Four to eight individuals of *L. semifasciata* and *L. laticaudata* were sampled at 2, 7, 14 days after the transfer (the FW groups were presented as FW2, FW7 and FW14 respectively; the SW groups were presented as SW2, SW7 and SW14). Five to eight individuals of *L. colubrina* were sampled at 2, 7 days after the transfer. The salinity of water was monitored by a refractometer (PAL-06S, Atago , Tokyo, Japan). The animals were not fed and the water temperature was kept at 27°C with a 12 h light: 12 h dark photoperiod during experimental period. In

this study, all animals were treated in accordance with a protocol approved by The Animal Care and Use Committee of National Taiwan Normal University (permit #93013).

Tissue sampling

The animals were euthanized by decapitation and their blood was collected from the wound with microtubes. The blood was for the analysis of plasma osmolality, ionic concentrations and hematocrit. The posterior sublingual glands and kidneys were excised and stored at -80°C for less than 2 month before Na^+/K^+ -ATPase activity measurement. For the Na^+/K^+ -ATPase activity measurement, approximately 0.3 g of tissues was taken from anterior kidney and whole salt gland tissues were used. The muscles of lateral trunk were excised for water content measurement immediately.

Measurement of plasmas osmolality, ionic concentration and hematocrits

The blood was centrifuge at 6000 ***g*** for 5 min immediately, and aliquots of plasma were stored at -20°C for the analysis of plasma osmolality and ionic concentrations. The plasma osmolality was

determined with a vapor pressure osmometer (Wescor Model 5520, Logan, UT , USA), and Na^+, Cl^-, K^+ concentration were determined by a biochemistry analyzer (Vitro 5.1 FS, Johnson &Johnson, NJ, USA) at Chiu Hospital, Taipei. The 1 ml blood were mixed with 10μl (0.5mg ml^{-1}) heparin (Sigma, St Louis, MO, USA) before hematocrit measurement. The hematocrit (ratio of corpuscle volume to blood volume) was determined by centrifuging 1 ml blood at 13000 ***g*** for 10 min at 4°C.

Water content of muscle

Surface water and blood on the muscle tissues were cleaned with tissue papers. The water content was determined by calculating the percentage of weight loss after drying the tissues at 100 °C for 3 days (Huang et al., 2010).

Protein extraction from salt glands and kidneys

The salt gland tissues or kidneys were homogenized in a homogenizing medium [25 mM Tris-HCl (Bio-Rad, Hercules, CA, USA), 0.25 mM sucrose (Mecrk, Darmstadt, Germany), 20 mM EDTA (Sigma, St Louis, Mo, USA), 0.4% sodium deoxycholate (Sigma, pH 7.4)] which

containing protease inhibitors [3.31 mM antipain (Sigma), 2.16 mM leupeptin (Sigma) and 63.86 mM benzamidine (Sigma) in aprotinin saline solution (5–10 trypsin inhibitor unit ml^{-1}, Sigma)] by ultrasonic processor. The protease inhibitor and homogenizing medium volumetric ratio was 1:200. The homogenats were first centrifuged at 4°C, 6000 ***g*** for 15min and then centrifuged at 4°C, 20000 ***g*** for 20min. The fresh supernatants were immediately analyzed for NKA activity. Then, 5 μL of supernatant was further diluted to 50 μL with deionized water. An aliquot of 10 μL of this mixture was further diluted to 800 μL with deionized water. This diluted supernatant was mixed well with 200 μL of protein assay solution (Bio-Rad). Total protein levels were determined by a spectrophotometer (U-2001; Hitachi, Japan) at a wavelength of 595 nm.

Na^+/K^+ -ATPase activity of PSG (salt gland) and kidney measurement

The method of measurement of NKA activity was based on the protocol developed by Tasi and Lin (Tasi and Lin, 2007) with slight modification. Briefly, NKA activity measurement was assayed in NKA inhibitor oubain-contained reaction medium [20 mM imidazole (Sigma), 130 mM NaCl (Mecrk), 10 mM $MgCl_2$ (Mecrk), 1 mM oubain (Sigma), pH 7.4] and oubain-free reaction medium [20 mM imidazole, 100 mM NaCl, 30mM KCl (Mecrk), 10 mM $MgCl_2$, pH 7.4], each containing the tissue (salt gland or kidney) supernatant. The mixture was added 100μl

of ATP stock solution (25 mM Na_2ATP) and incubated at 37°C for 15 min. The reaction was stopped by adding of 200µl of ice-cold TCA stock solution (30% trichloroacetic acid). The reaction mixture was centrifuged at a speed of 1640 ***g***, 4°C, for 10 min and the supernatant (500 µl) was collected. The NKA activity was calculated as difference in the inorganic phosphates (Pi) content between oubain-contained and oubain-free reaction solution. The Pi concentration was determined by Boting's color reagent [560mM H_2SO_4 (Sigma), 8.1mM ammonium molybdate tetrahydate (Sigma), 176 mM $FeSO_4$ (Sigma)] with a spectrophotometer. Ice-cold Boting's color reagent (1000 µL) was added to the supernatant (500 µL), incubated at 20°C water bath for 20 min, and the absorbance of the solution was measured at 700 nm. NKA activity was expressed as µmol Pi mg^{-1} protein h^{-1}.

Statistical analysis

All values are presented as mean ± SEM. All values were analyzed by one-way ANOVA and the Tukey *post-hoc* test. All analyses were conducted using JMP 7 (SAS Institute Inc. Cary, NC, USA). A value was considered statistically different when p value was less than 0.05 ($p < 0.05$).

Results

Animals

In the present study, 44 individuals of *L. semifasciata* (SVL = 91.2 ± 0.9 cm, mass = 512.0 ± 13.7 g, *N*=44), *L. laticaudata* (SVL = 81.5 ± 0.8 cm, mass = 159.2 ± 4.5 g, *N*=44) and 30 individuals of *L. colubrina* (SVL = 78.4 ± 1.0 cm, mass = 193.5 ± 8.4 g, *N*=30) were used.

Plasma osmolality

In *L. semifasciata,* the plasma osmolality was 301.9 ± 3.0 mOsm Kg^{-1} before transfer (FW0/ SW0). After transfer, the plasma osmolality of SW groups (309~311 mOsm Kg^{-1}) tend to be higher than that of FW groups (298~309 mOsm Kg^{-1}), however, the differences were not significant ($F_{6,37}$=1.8491, *P*=0.1162, ANOVA, Fig. 1A).

In *L. laticaudata,* the plasma osmolality was 302.4 ± 2.5 mOsm Kg^{-1} (FW0/ SW0) before transfer, and there is significantly different among all groups ($F_{6,37}$=11.3298, *P*<0.05, ANOVA, Fig. 1B). However, it increased significantly at 7 days (324.1 ± 2.6 mOsm Kg^{-1}, SW7) and 14 days (327.8 ± 2.6 mOsm Kg^{-1}, SW14) in SW (*P*<0.05, Tukey HSD, Fig. 1B), but not significantly changed in FW.

In *L. colubrina*, the plasma osmolality (332.4 ± 1.1 mOsm Kg^{-1}, FW0/ SW0) was higher than that in the other two species before transfer. There is significantly different among all groups ($F_{4,21}$=11.3298, *P*<0.05,

ANOVA, Fig. 1C). It decreased slightly after transfer to FW, and increased slightly after transfer to SW, but the changes were not significant (Fig. 1C). However, the SW7 group (339.2 ± 3.4 mOsm Kg^{-1}) was significantly higher than the FW7 (325.7 ± 1.2 mOsm Kg^{-1}) group (P<0.05, Tukey HSD, Fig. 1C).

Plasma ionic concentrations

In *L. semifasciata,* the plasma [Na^+] was 148.6 ± 2.0 mM (FW0/ SW0) before transfer, and it slightly increased after transfer to SW, and not significantly changed after transfer to FW (Fig. 2A). Similar changes were found in plasma [Cl^-]. It was 117.5 ± 1.6 mM (FW0/ SW0) before transfer, and it slightly increased after transfer to SW, and no significant change was found after transfer to FW (Fig. 3A). The plasma [K^+] was 4.4~4.8 mM (Fig. 4A). After transfer to either FW or SW, no significantly change was found in the plasma [Na^+], [Cl^-] and [K^+] (Na^+, $F_{6,37}$=1.6896, P=0.1510; Cl^-, $F_{6,34}$=2.1742, P=0.0700; K^+, $F_{6,37}$=0.9612, P=0.4646, ANOVA, Fig. 2A, 3A, 4A).

In *L. laticaudata*, the changes of plasma [Na^+] and [Cl^-] were similar. They slightly increased after transfer to SW and slightly decreased after transfer to FW. The plasma [Na^+] was 156.8 ± 1.9 mM (FW0/ SW0) before transfer, and the SW7 group (161.3 ± 1.2 mM) was significant higher than FW7 (154.1 ± 1.5 mM) group ($F_{6,37}$=1.6896, P<0.05, ANOVA; P<0.05, Tukey HSD, Fig. 2B). The Plasma [Cl^-] was 118.8 ± 3.4 mM (FW0/ SW0) before transfer, and the SW7 (125.9 ± 1.1 mM) and SW14

(125 ± 1.1 mM) groups were significant higher than the FW7 (117 ± 1.6 mM) and FW14 (116 ± 2.2 mM) groups ($F_{6,36}$=5.7848, P<0.001, ANOVA; P<0.05, Tukey HSD, Fig. 3B). The plasma [K^+] was 4.2 ~ 4.6 mM (Fig. 4B). No significant difference was found in plasma [K^+] after transfer to FW and SW (K^+, $F_{6,37}$=1.2707, P=0.2944, ANOVA, Fig 4B).

In *L. colubrina*, plasma [Na^+] was 162.2 ± 1.4 mM (FW0/ SW0) before transfer, and no significant changes was found after transfer to FW or SW ($F_{4,24}$=2.3934, P=0.0787, ANOVA, Fig. 2C). The plasma [Cl^-] of *L. colubrina* was 120.2 ± 1.8 mM (FW0/ SW0, N=5) before transfer. After transfer to SW, it slightly increased at 2 days and significantly increased at 7days (127.7 ± 1.4 mM, SW7; $F_{4,24}$=4.7683, P<0.05, ANOVA; P<0.05, Tukey HSD, Fig. 3C). The SW7 (127.7 ± 1.4 mM) group was significant higher than FW7 (121.7 ± 0.6 mM) group (P<0.05, Tukey HSD, Fig. 3C). The plasma [K^+] was 5.3 ~ 5.7 mM (Fig. 4C). No significant difference was found after transfer to FW or SW (K^+, $F_{4,24}$=1.2171, P=0.3296, ANOVA, Fig. 4C).

Hematocrit and muscular water content

Hematocrit of *L. semifasciata* was 41.5~47.2% and higher than the other two species (Table 1). *L. laticaudata* was 36.0~40.2% and lower than *L. semifasciata* (Table 1). *L. colubrina* was 33.4~36.4% and lower than the other species (Table 1). In the three species, no significant differences was found in hematocrit after transfer to FW and SW (*L.*

semifasciata, $F_{6,36}$=0.7306, *P*=0.6281; *L. laticaudata*, $F_{6,36}$=0.6201, *P*=0.7129; *L. colubrina*, $F_{4,22}$=0.7614, *P*=0.5615, ANOVA, Table 1).

In *L. semifasciata*, the muscular water content was 81.9 ± 0.9 % (FW0/ SW0) before transfer. No significant change was found after transfer to FW or SW ($F_{4,28}$=1.1510, *P*=0.3533, ANOVA, Fig. 5A).

In *L. laticaudata*, the muscular water content was 78.7 ± 0.5 % (FW0/ SW0) before transfer, and it slightly increased after transfer to SW and slightly increased after transfer to FW (Fig. 5B). The SW2 (79.6 ± 0.4 %) and FW2 (80.1 ± 0.3 %) groups were significantly higher than SW7group (78.0 ± 0.3 %; $F_{4,26}$=5.0690, *P*<0.05, ANOVA; *P*<0.05, Tukey HSD, Fig. 5B).

Similar to *L. laticaudata*, muscular water content of *L. colubrina* was 78.6 ± 0.3 % (FW0/ SW0, *N*=5) before transfer. After transfer to FW or SW, the muscular water content of SW groups tend to be higher than that of FW groups, and the FW2 group (80.1 ± 0.3 %) was significantly higher than SW7 (78.6 ± 0.4) % group ($F_{4,23}$=3.6476, *P*<0.05, ANOVA; *P*<0.05, Tukey HSD, Fig. 5C).

Na^+/K^+ -ATPase activity of PSG (salt gland)

In *L. semifasciata*, initial NKA activity of PSG was relatively higher than that in the other species (31.6 ± 3.3 μmol Pi mg^{-1} protein h^{-1}, FW0/ SW0, *N*=8). There is significantly different among all groups ($F_{6,34}$=3.5297, *P*<0.05, ANOVA, Fig. 6A). No significantly change was

found after transfer to SW (Fig. 6A). After transfer to FW, it slightly decreased at 2 days and significantly decreased at 7 days (17.0 ± 1.7 μmol Pi mg^{-1} protein h^{-1}, FW7, Tukey HSD, Fig. 6A). However, it increased significantly at 14 days (27.5 ± 0.9 μmol Pi mg^{-1} protein h^{-1}, FW14; $P<0.05$, Tukey HSD).

In contrast, initial NKA activity of PSG in *L. laticaudata* was relatively low (7.1 ± 0.5 μmol Pi mg^{-1} protein h^{-1}, FW0/ SW0), and there is significantly different among all groups ($F_{6,34}= 3.9753$, $P<0.05$, ANOVA, Fig. 6B). No significant difference was found after transfer to FW (Fig. 6B). After transfer to SW, NKA activity of PSG significantly increased at 7 days (12.5 ± 1.6 μmol Pi mg^{-1} protein h^{-1}, SW7; $P<0.05$, Tukey HSD, Fig. 6B). And then it decreased significantly at 14 days (6.2 ± 0.5 μmol Pi mg^{-1} protein h^{-1}, SW14; $P<0.05$, Tukey HSD).

In *L. colubrina*, the NKA activity of PSG was 11.6 ± 1.1 μmol Pi mg^{-1} protein h^{-1} (FW0/ SW0, $N=5$) before transfer. However, no significant difference was found after transfer to FW or SW ($F_{4,23}= 1.3653$, $P=0.2767$, ANOVA, Fig. 6C).

Na^+/K^+ -ATPase activity of kidney

NKA activity in kidney was apparently lower than that in salt gland. In *L. semifasciata,* it was 3.1~3.8 μmol Pi mg^{-1} protein h^{-1}. In *L. laticaudata* it was 3.4~5 μmol Pi mg^{-1} protein h^{-1}. In *L. colubrina,* it

was 3.3~5.1μmol Pi mg^{-1} protein h^{-1} (Table 2). No significant difference was found among all groups in the three species (*L. semifasciata*, $F_{6,34}$: 0.2671, *P*=0.9485; *L. laticaudata*, $F_{6,37}$: 1.7340, *P*=0.1404; *L. colubrina*, $F_{4,23}$: 1.9585, *P*=0.1347, ANOVA, Table 2).

Discussion

Water and salt balance

L. semifasciata maintained plasma osmolality and ionic concentrations relatively constant after transfer to FW or SW, whereas *L. laticaudata* and *L. colubrina* showed significant or slightly changes after transfer. In *L. laticaudata*, plasma [Na^+], [Cl^-] and osmolality were relatively low before transfer, and they increased after transfer to SW. In *L. colubrina*, plasma osmolality was relatively high and only slightly increased after transfer to SW and slightly decreased after transfer to FW. These results suggest that the three sea kraits have different capabilities or strategies to respond to osmotic challenges. *L. semifasciata* showed better capability in osmoregulation than the other two species. There is a correlation between their physiological properties and ecological properties in the three sea kraits. The higher marine tendency species (*L. semifasciata*) has better capability in osmoregulation. Similar comparative studies were reported in fishes such as three salmonids (Hiroi and McCormick, 2007) and two gobiids (Rigal et al., 2008). Hiroi and McCormick (2007) transferred three salmonids from FW to SW and found that the anadromous fish, *Salmo salar*, maintained their plasma [Na^+] and [Cl^-] relatively constant than other two species, *Salvelinus namaycush* and *S. fontinalis* which display a more restricted pattern of seaward migration. *S. namaycush* is a non-anadromous species and is restricted to cold freshwater lakes. *S. fontinalis* is mostly

non-anadromous and only some anadromous population presents in the northern part of their distribution (non-anadromous population fish was used in that study). Rigal et al. (2008) reported that two gobiid, *Pomatoschistus microps* and *P. marmoratus,* have different omsoregulatory capacity. *P. marmoratus* is distributed in Thau lagoon of French, and *P. microps* is distributed in Mauguio lagoon of French. The salinity of Thau lagoon is relatively stable (31 ~ 38 ppt), and the salinity of Mauguio lagoon is from 0.1 ~ 37 ppt. The authors acclimated the two gobiid to various salinity environments and found that *P. microps* performed better osmoregulation than *P. microps*. Different salt excretion capability was also reported in crocodilians (Taplin et al., 1982) and ducks (Bennet and Hughes 2003). Taplin et al. (1982) compared lingual salt secretions in three crocodylids and two alligatorids after methacholine injection. The concentration and rate of salt secretions in *C. porosus* and *C. acutus* were higher than that in *C. johnstoni*. The *C. porosus*, *C. acutus* are distribution in saline habitat, while *C. johnstoni* is restricted to FW and occasionally in saline water. Bennett and Hughes (2003) examined and compared the salt secretions in three ducks after saline infusions. Barrow's goldeneyes (*Bucephala islandica*), the most marine species, have the highest rate of salt secretion than the other two species which are freshwater and estuarine species. In addition, only Barrow's goldeneyes can secrete all the infused salt *via* their salt glands.

Compared to teleosts, the changes of plasma osmolality and ionic concentrations in sea kraits subjected to salinity changes are relatively

small. The changes of plasma osmolality in teleost are 50 ~ 100 mOsm Kg^{-1} and plasma [Na^+] and [Cl^-] are 25 ~ 100 mM (Chew et al., 2009; Jensen et al., 1998; Huang et al., 2010; Kelly and Woo, 1999; Lin et al., 2006; Scott et al., 2006; Lin et al., 2004a; Lin et al., 2004b). In this study, the maximal change of plasma osmolality is 20 mOsm Kg^{-1} and plasma [Na^+] and [Cl^-] are 10 mM in the sea kraits. In euryhaline teleosts, the problem of osmotic and salt gradients is exacerbated by the presence of gill epithelium. This large and thin tissue is modified for efficient gas exchange. However, this property also makes the gills to be the site for passive movements of both water and salt (Evans et al., 2005). To balance the water loss, teleosts have to drink hypertonic sea water and it may cause the increase of plasma osmolality. In contrast, the sea kraits are air breathers and do not use gills as respiratory organs. Their skin is slightly permeable to water but not Na^+ (Dunson and Robinson, 1976; Lillywhite et al., 2009). Therefore, the minor changes in plasma osmolality and ionic concentrations may be due to limited water loss and salt gain from skin or guts.

Interestingly, the plasma osmolality change in *L. colubrina* is mainly caused by plasma [Cl^-] but not [Na^+] (Fig. 1, 2, 3). However, the plasma osmolality changes of *L. semifasciata* and *L. laticaudata* are mainly caused by both plasma [Na^+] and [Cl^-] (Fig. 1, 2, 3). In euryhaline teleosts subjected to salinity change, their plasma osmolality changes are usually caused by both Na^+ and Cl^- changes (Hiroi and McCormick, 2007; Jensen et al., 1998; Lin et al., 2006; Scott et al., 2006) or only Cl^- changes (Kelly and Woo, 1999; Lin et al., 2004a; Lin et al., 2004b). This

phenomenon indicates that Na^+ and Cl^- may be independently regulated in these euryhaline vertebrates.

Muscular water content can be used as an indicator for osmoregulatory capacity in teleost (Freire et al., 2008; Kelly et al., 1999; Wood and Chung, 1995). In addition, the decrease of muscular water content was accompanied by the increase of plasma osmolality in some teleosts acclimated to SW (Huang et al., 2010; Tipsmark et al., 2002). In this study, the most marine species (*L. semifasciata*) maintained water content more stable than the other two species during SW acclimation. In *L. laticaudata* and *L. colubrina*, the water content was relatively low after transferred to SW for 7 days, suggesting that the osmoregulatory capability of these two species was not as good as that of *L. semifasciata*. Take plasma osmolality, $[Na^+]$, $[Cl^-]$ and muscular water content together, these data suggest different osmoregulatory capability among the three sea kraits. However, no significant difference was found in hematocrit among the three species. Rebecca et al. (2008) reported that after 6 month SW or FW acclimation, the plasma osmolality and hematocrit of estuarine crocodile (*C. porosus*) was significantly higher in SW than FW. But the hematocrit values were close between SW and FW group (P=0.042). Pillans et al. (2005) reported that the plasma osmolality of bull sharks (*Carcharhinus leucas*) was higher in SW than in FW, but no significant difference was found in the hematocrit. Therefore, hematocrit may not be a sensitive parameter to reflect the water and salt balance in animals.

Taken those data together, the most marine species, *L. semifasciata*,

showed best ability to maintain salt and water balance than the other two species.

Na^+/ K^+ ATPase (NKA) activity

Na^+/K^+ ATPase (NKA) plays a critical role in salt secretion of MR cells in SW teleost gills. NKA is located in the basolateral membrane of MR cells to create Na^+ gradient across cell membrane. The Na^+ gradient drives a basolateral NKCC to carry Na^+, K^+ and Cl^- into MR cell. Accumulated Cl^- leaves MRC through an apical CFTR chloride channel (Marshall, 2002; Evans et al., 2005). It has been well documented that euryhaline teleosts regulate branchial NKA activity during salinity changes (Marshall, 2002; Evans et al., 2005). In some euryhaline teleosts such as salmon, eel, tilapia and spotted green pufferfish, branchial NKA activity increase after transfer from hypotonic to hypertonic environments, suggesting that NKA is required for salt secretion (Lin et al., 2004a; Lin et al., 2004b; Tipsmark et al., 2002; Wilson et al., 2007). The mechanism of salt secretion by salt glands is similar to that in teleost gill. In euryhaline elasmobranch, the NKA activity of rectal (salt) gland was significant higher in SW than in FW (Piermarini and Evans, 2000; Pillans et al., 2005). In this study, the NKA activity was higher in SW than in FW in *L. semifasciata* and *L. laticaudata*, suggesting that NKA is involved in salt secretion by PSG. Interestingly, the NKA activity of PSG showed differential responses to salinity changes among the three sea kraits. In *L. semifasciata*, the NKA

activity was relatively high before transfer, and decreased after transfer to FW at 7 days, indicating that the NKA activity is downregulated in hypotonic environment. Interestingly, the NKA activity was restored to the level before transfer. It might be due to a stress response caused by a long time of starvation (at least 21 Days) or unknown factors. In contrast, no significant change was found in the NKA activity after transfer to SW. The NKA activity was relatively high before transfer, therefore it might be high enough to cope with SW acclimation without upregulation of NKA activity.

In *L. laticaudata*, the NKA activity increased after transfer to SW. However, their plasma osmolality and ionic concentration increased in SW, suggesting that their osmoregulatory capability is not as good as *L. semifasciata*. After transferred to SW for 14 days, the NKA activity returned to lower level. I found that these animals became inactive and weak after 14 days of SW acclimation. Therefore, the decrease of NKA activity might be due to an insufficient energy supply. In addition, evidence suggests that water permeability of skin increases in terrestrial vertebrates acclimating to humidity environment (Lillywhite, 2007). Therefore, *L. laticaudata* might decrease skin permeability to water after 14 days SW acclimation to decrease energy cost in NKA activity.

In *L. colubrina*, the NKA activity did not significantly change after SW or FW transfer. However, their plasma omsolality and ionic concentration were relatively constant during SW and FW acclimation. Since this species has lower water permeability in their skin, they might be also to maintain their plasma osmolality constant without regulate the

activity of salt glands.

The NKA activity of kidney was significantly lower than that in PSG, and the activity was not responding to salinity changes, suggesting that the kidney of sea kraits may not play a critical role in salt secretion.

Osmoregulations in three sea kraits

Lillywhite et al (2009) reported that the skin permeability to water of *L. semifasciata* was the highest, *L. laticaudata* was in-between and *L. colubrina* was the lowest. The skin permeability to water is associated with dehydration rate in the air, and it was lower in *L. colubrina* than in the other two species (Lillywhite et al., 2008). On the contrary, when they are in SW, the dehydration rate of *L. colubrina* was higher than that of the other two species (Lillywhite et al., 2008). It is not clear why the dehydration rate was opposite in SW and in the air? It might be due to that *L. semifasciata* and *L. laticaudata* drink SW but *L. colubrina* does not. Since the NKA activity of PSG in *L. semifasciata* and *L. laticaudata* were different in FW and SW, but *L. colubrina* was not. To test this possibility, in the future we need to quantify the drinking rate of the sea kraits in SW (Scott et al., 2006).

Conclusion

1. Osmoregulatory properties among three sea kraits are correlated with their habitat affinities. Higher marine tendency species has better capability in osmoregulation.
   (a) The capability of osmoregulation is better in *L. semifasciata* than the other two species.
   (b) *L. colubrina* may have different osmoregulatiory strategy with *L. semifasciata* and *L. laticaudata*.
2. In *L. semifasciata* and *L. laticaudata*, Na^+/K^+ ATPase is regulated in response to salinity acclimation.

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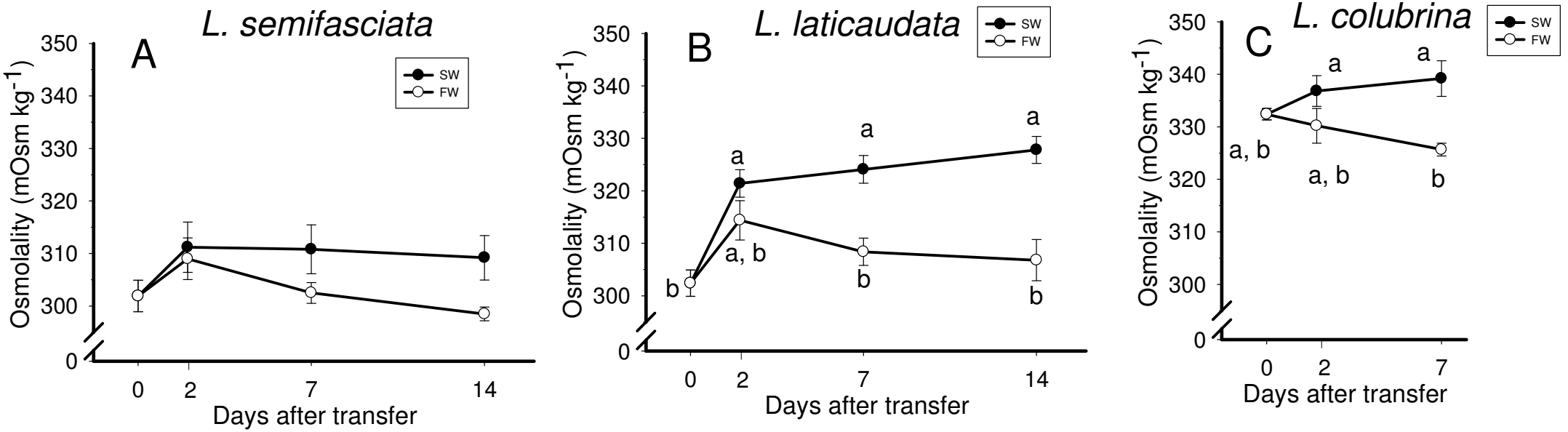

Fig. 1. Time course changes of plasma osmolality in three sea kraits transferred from land to sea water (SW; filled circles) or fresh water (FW; open circles). Each value represents the mean ± SEM. Sample size in *L. semifasciata*: FW0/SW0, 8; FW2, 5; FW7, 8; FW14, 5; SW2, 5; SW7, 8; SW14, 5. Sample size in *L. laticaudata*: FW0/SW0, 8; FW2, 5; FW7, 8; FW14, 5; SW2, 5; SW7, 8; SW14, 5. Sample size in *L. colubrina*: FW0/SW0, 5; FW2, 5; FW7, 6; SW2, 5; SW7, 5. Values with different lowercase letters are significantly different (P<0.05, Tukey HSD).

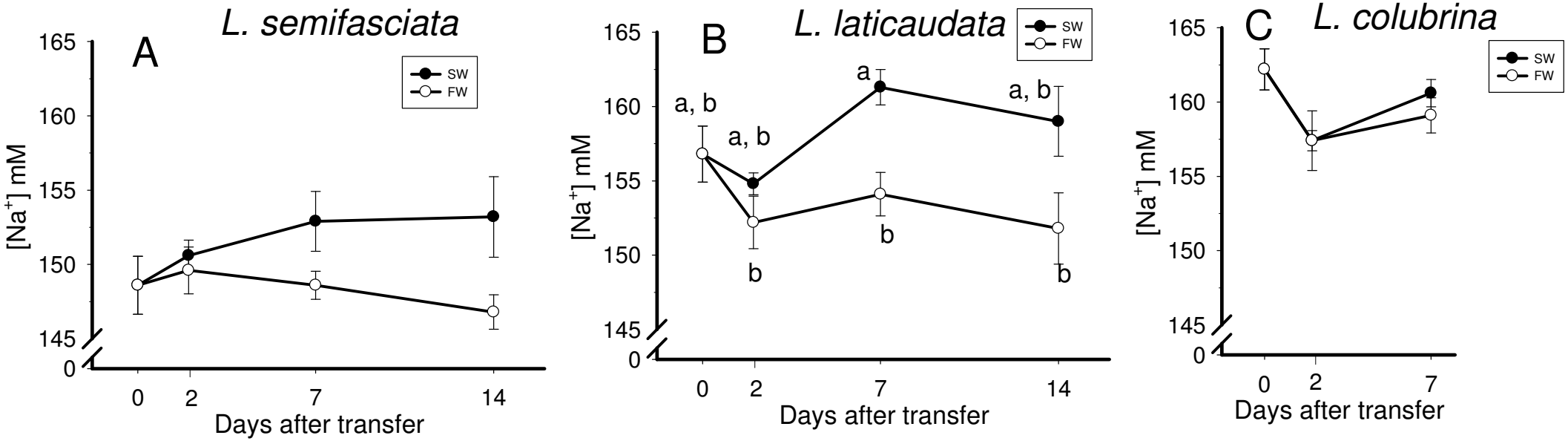

Fig. 2. Time course changes of plasma [Na^+] in three sea kraits transferred from land to sea water (SW; filled circles) and to fresh water (FW; open circles). Each value represents the mean ± SEM. Sample size in *L. semifasciata*: FW0/SW0, 8; FW2, 5; FW7, 8; FW14, 5; SW2, 5; SW7, 8; SW14, 5. Sample size in *L. laticaudata*: FW0/SW0, 8; FW2, 5; FW7, 8; FW14, 5; SW2, 5; SW7, 8; SW14, 5. Sample size in *L. colubrina*: FW0/SW0, 5; FW2, 5; FW7, 7; SW2, 5; SW7, 7. Values with different lowercase letters are significantly different (P<0.05, Tukey HSD).

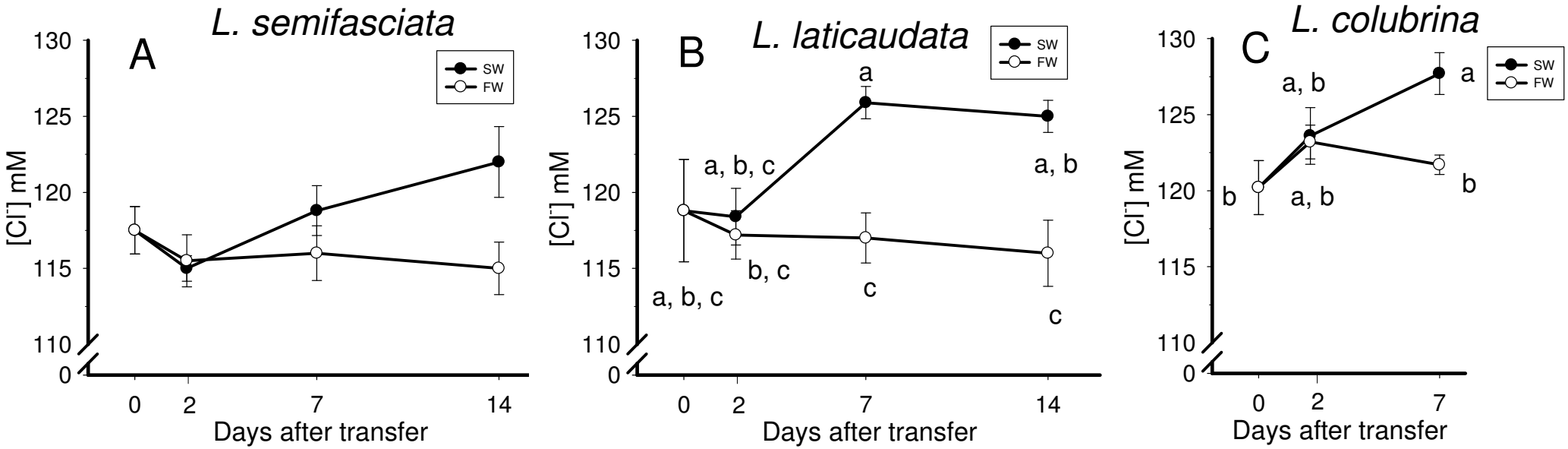

Fig. 3. Time course changes of plasma [Cl^-] in three sea kraits transferred from land to sea water (SW; filled circles) and to fresh water (FW; open circles). Each value represents the mean ± SEM. Sample size in *L. semifasciata*: FW0/SW0, 8; FW2, 4; FW7, 8; FW14, 5; SW2, 5; SW7, 6; SW14, 5. Sample size in *L. laticaudata*: FW0/SW0, 7; FW2, 5; FW7, 8; FW14, 5; SW2, 5; SW7, 8; SW14, 5. Sample size in *L. colubrina*: FW0/SW0, 5; FW2, 5; FW7, 7; SW2, 5; SW7, 5. Values with different lowercase letters are significantly different (P<0.05, Tukey HSD).

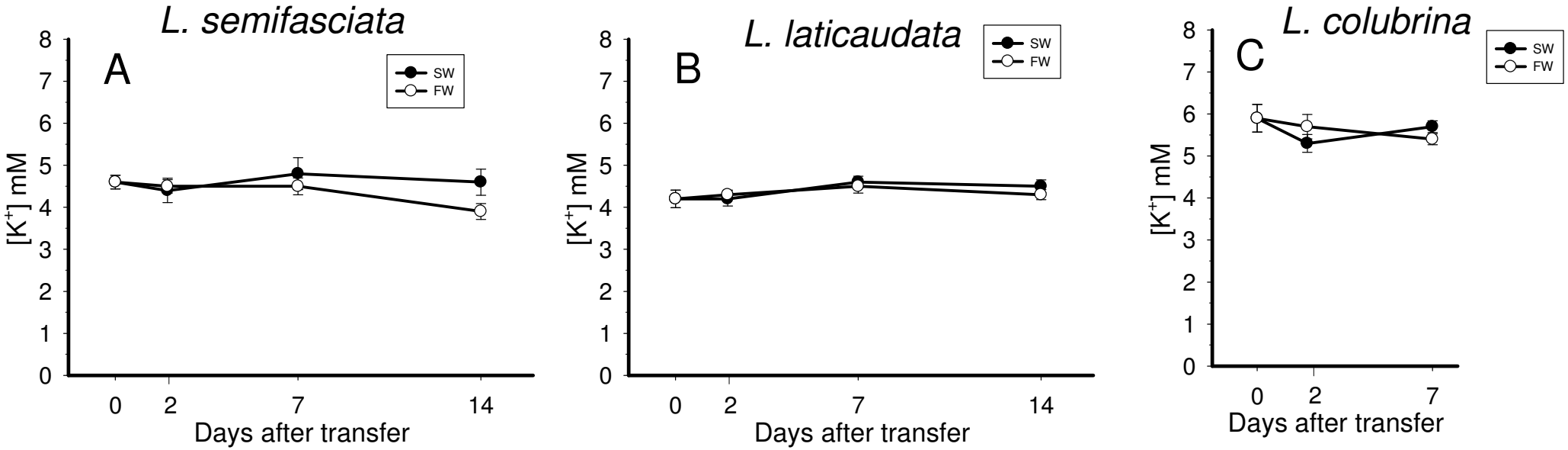

Fig. 4. Time course changes of plasma $[K^+]$ in three sea kraits transferred from land to sea water (SW; filled circles) and to fresh water (FW; open circles). Each value represents the mean ± SEM. Sample size in *L. semifasciata*: FW0/SW0, 8; FW2, 5; FW7, 8; FW14, 5; SW2, 5; SW7, 8; SW14, 5. Sample size in *L. laticaudata*: FW0/SW0, 8; FW2, 5; FW7, 8; FW14, 5; SW2, 5; SW7, 8; SW14, 5. Sample size in *L. colubrina*: FW0/SW0, 5; FW2, 5; FW7, 7; SW2, 5; SW7, 7.

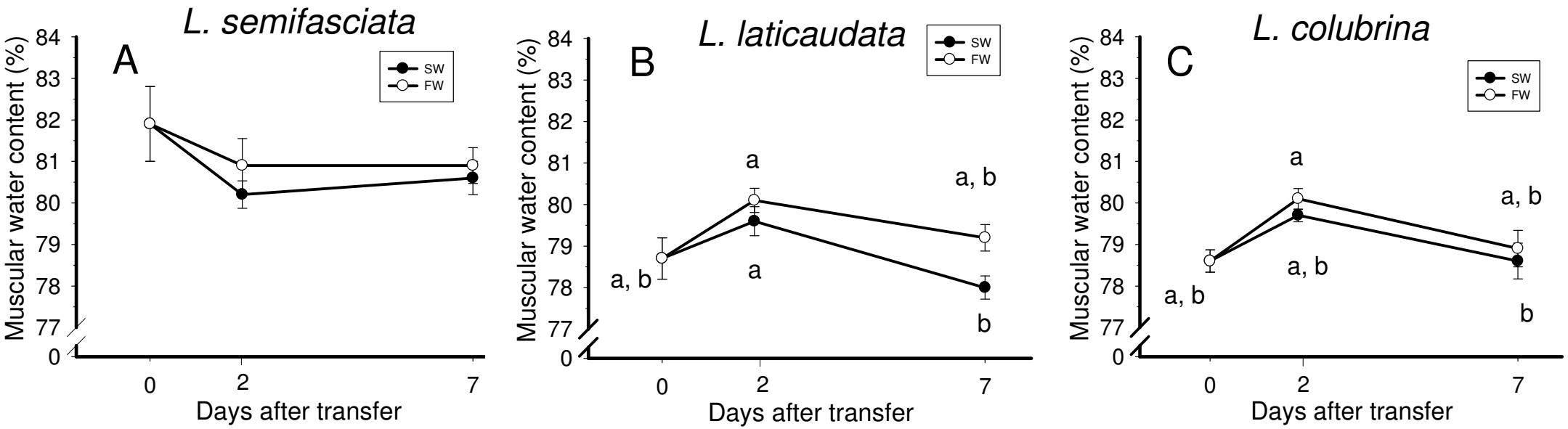

Fig. 5. Time course changes of muscular water content in three sea kraits transferred from land to sea water (SW; filled circles) and to fresh water (FW; open circles). Each value represents the mean ± SEM. ample size in *L. semifasciata*: FW0/SW0, 7; FW2, 5; FW7, 8; SW2, 5; SW7, 8. Sample size in *L. laticaudata*: FW0/SW0, 7; FW2, 5; FW7, 6; SW2, 5; SW7, 8. Sample size in *L. colubrina*: FW0/SW0, 5; FW2, 5; FW7, 7; SW2, 5; SW7, 6. Values with different lowercase letters are significantly different (P<0.05, Tukey HSD).

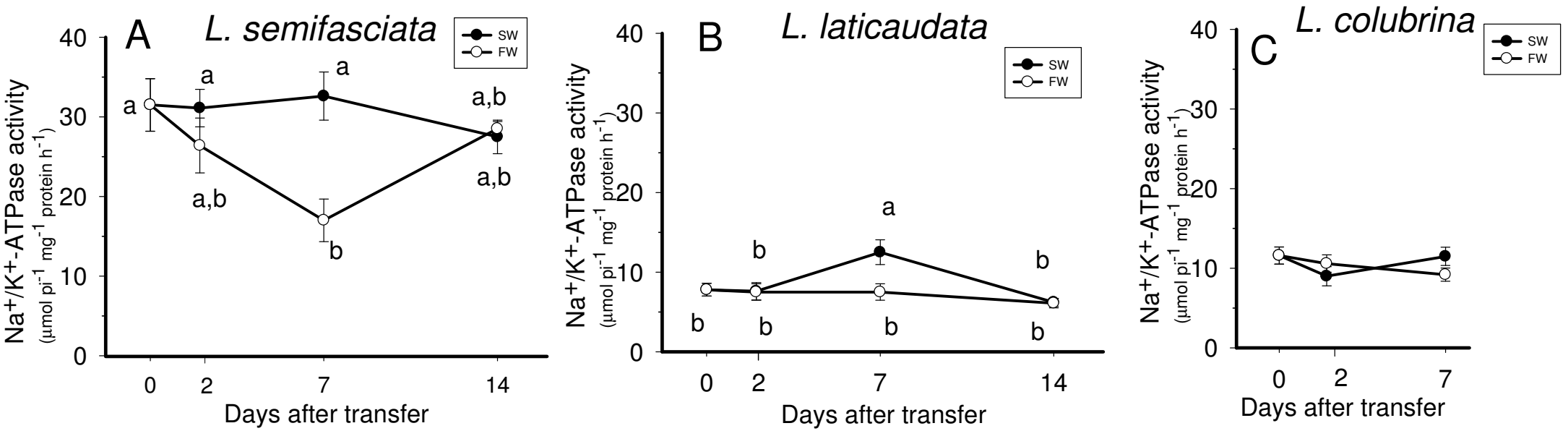

Fig. 6. Time course changes of NKA activity of PSG in three sea kraits transferred from land to sea water (SW; filled circles) and to fresh water (FW; open circles). Each value represents the mean ± SEM. Sample size in *L. semifasciata*: FW0/SW0, 8; FW2, 5; FW7, 6; FW14, 5; SW2, 5; SW7, 7; SW14, 5. Sample size in *L. laticaudata*: FW0/SW0, 7; FW2, 5; FW7, 7; FW14, 5; SW2, 5; SW7, 8; SW14, 5. Sample size in *L. colubrina*: FW0/SW0, 5; FW2, 5; FW7, 7; SW2, 5; SW7, 6. Values with different lowercase letters are significantly different. (P<0.05, Tukey HSD).

Table 1. Time course changes of hematocrit (%) in three sea kraits transferred from land to sea water and to fresh water.

Species	Treatment condition							*P* -value
	FW0/ SW0	FW2	FW7	FW14	SW2	SW7	SW14	
L. semifasciata	44.6± 1.8(7)	45.4± 3.2(5)	43.4± 10.0(8)	47.2± 1.4(5)	43.4± 0.9(5)	41.5± 2.8(8)	45.0± 2.1(5)	0.6281
L. laticaudata	37.1± 1.7(7)	36.0± 1.3(5)	38.3± 1.5(8)	38.8± 1.0(5)	38.0± 1.5(5)	36.9± 1.2(8)	40.2± 2.9(5)	0.7129
*L. colubrin*a	36.4± 1.3(5)	33.4± 1.3(5)	34.0± 1.2(7)	-	33.4± 1.9(5)	34.8± 1.2(5)	-	0.5615

Values are means ± SEM (sample size).

Table 2. Time course changes of NKA activity of kidney (μmol Pi^{-1} mg^{-1} h^{-1}) in three sea kraits transferred from land to sea water and to fresh water.

	Treatment condition							
Species	FW0/ SW0	FW2	FW7	FW14	SW2	SW7	SW14	*P* -value
L. semifasciata	3.4 ± 0.4(7)	3.5± 0.5(5)	3.5± 0.7(6)	3.7± 0.6(5)	3.8± 0.5(5)	3.1± 0.4(8)	3.8± 0.5(5)	0.9485
L. laticaudata	3.4± 0.6 (8)	5.0± 0.6(5)	5.0± 0.4 (8)	3.6± 0.8(5)	5.8± 0.5(5)	4.4± 0.5(8)	4.3± 0.4(5)	0.1404
*L. colubrin*a	5.1± 0.5 (5)	4.5± 0.2(5)	3.5± 0.5 (6)	-	4.3± 0.7(5)	3.3± 0.4(7)	-	0.0909

Values are means ± SEM (sample size).

附錄、三種闊尾海蛇資本資料

物種	編號	體重 (g)	全長(cm)	吻肛長(cm)	尾長(cm)	採集地點	性別	記錄日期	處理組別
LS	LS33	549.9	108	98	10	Orchid	male	2009.08.23	FW0/SW0
LS	LS34	491.5	102	90	12	Orchid	male	2009.08.23	FW0/SW0
LS	LS35	429.0	100	87	13	Orchid	male	2009.08.23	FW0/SW0
LS	LS36	504.9	114	92	22	Orchid	male	2009.08.23	FW0/SW0
LS	LS37	621.1	114	100	14	Orchid	male	2009.08.23	FW0/SW0
LS	LS38	610.5	108	97	11	Orchid	male	2009.08.23	FW7
LS	LS39	436.9	100	89	11	Orchid	male	2009.08.23	FW7
LS	LS40	326.5	86	75	11	Orchid	male	2009.08.23	FW7
LS	LS41	483.7	103	90	13	Orchid	male	2009.08.23	FW7
LS	LS42	721.8	115	104	11	Orchid	male	2009.08.23	FW7
LS	LS43	485.2	95	85	10	Orchid	male	2009.08.23	SW7
LS	LS44	619.6	108	95	13	Orchid	male	2009.08.23	SW7
LS	LS45	441.8	97	85	12	Orchid	male	2009.08.23	SW7
LS	LS46	496.6	106	94	12	Orchid	male	2009.08.23	SW7
LS	LS48	527.5	107	95	12	Orchid	male	2009.08.23	SW7
LS	LS49	496.1	104	92	12	Orchid	male	2009.09.16	FW14
LS	LS50	564.6	107	96	11	Orchid	male	2009.09.16	FW14

物種	編號	體重 (g)	全長(cm)	吻肛長(cm)	尾長(cm)	採集地點	性別	記錄日期	處理組別
LS	LS51	350.3	90	80	10	Orchid	male	2009.09.16	FW14
LS	LS52	417.0	98	89	9	Orchid	male	2009.09.16	FW14
LS	LS53	633.5	110	97	13	Orchid	male	2009.09.16	SW14
LS	LS55	360.1	87	77	10	Orchid	male	2009.09.16	SW14
LS	LS56	410.5	104	91	13	Orchid	male	2009.09.16	SW14
LS	LS57	389.5	100	89	11	Orchid	male	2009.09.16	SW14
LS	LS58	564.2	103	92	11	Orchid	male	2009.09.16	SW14
LS	LS59	386.9	93	83	10	Orchid	male	2009.09.16	FW14
LS	LS60	618.0	104	94	10	Orchid	male	2010.05.13	FW0/SW0
LS	LS61	417.9	100	87	13	Orchid	male	2010.05.28	FW0/SW0
LS	LS62	454.9	103	90	13	Orchid	male	2010.05.28	FW0/SW0
LS	LS63	608.1	112	98	14	Orchid	male	2010.05.28	FW2
LS	LS64	452.3	102	89	13	Orchid	male	2010.05.28	FW2
LS	LS65	534.7	115	100	15	Orchid	male	2010.05.28	FW2
LS	LS66	541.7	106	92	14	Orchid	male	2010.05.28	FW2
LS	LS67	658.8	112	97	15	Orchid	male	2010.05.28	FW2
LS	LS68	409.1	98	84	14	Orchid	male	2010.05.28	SW2
LS	LS69	607.3	114	100	14	Orchid	male	2010.05.28	SW2
LS	LS70	553.7	107	93	14	Orchid	male	2010.05.28	SW2
LS	LS71	513.7	101	89	12	Orchid	male	2010.05.28	SW2

物種	編號	體重(g)	全長(cm)	吻肛長(cm)	尾長(cm)	採集地點	性別	記錄日期	處理組別
LS	LS72	544.6	108	93	15	Orchid	male	2010.05.28	SW2
LS	LS73	513.1	101	90	11	Orchid	male	2010.05.28	FW7
LS	LS74	492.4	98	84	14	Orchid	male	2010.05.28	FW7
LS	LS75	568.2	107	93	14	Orchid	male	2010.05.28	FW7
LS	LS76	586.0	109	95	14	Orchid	male	2010.05.28	SW7
LS	LS77	631.7	110	97	13	Orchid	male	2010.05.28	SW7
LS	LS78	531.1	106	94	12	Orchid	male	2010.05.28	SW7
LL	LL17	147.8	87	77	10	Orchid	male	2009.08.22	FW0/SW0
LL	LL18	134.8	90	79	11	Orchid	male	2009.08.22	FW0/SW0
LL	LL19	200.5	96	87	9	Orchid	male	2009.08.22	FW0/SW0
LL	LL20	143.3	88	79	9	Orchid	male	2009.08.22	FW7
LL	LL21	169.0	94	85	9	Orchid	male	2009.08.22	FW0/SW0
LL	LL22	147.8	94	84	10	Orchid	male	2009.08.22	FW0/SW0
LL	LL23	203.6	92	81	11	Orchid	male	2009.08.22	FW7
LL	LL24	116.8	82	72	10	Orchid	male	2009.08.22	FW7
LL	LL25	126.3	88	78	10	Orchid	male	2009.08.22	FW7
LL	LL26	128.0	88	78	10	Orchid	male	2009.08.22	FW7
LL	LL27	162.9	93	82	11	Orchid	male	2009.08.22	SW7
LL	LL28	129.0	87	77	10	Orchid	male	2009.08.22	SW7
LL	LL29	186.0	97	86	11	Orchid	male	2009.08.22	SW7

物種	編號	體重(g)	全長(cm)	吻肛長(cm)	尾長(cm)	採集地點	性別	記錄日期	處理組別
LL	LL30	127.2	84	74	10	Orchid	male	2009.08.22	SW7
LL	LL31	194.0	100	90	10	Orchid	male	2009.08.22	SW7
LL	LL32	220.9	97	86	11	Green	male	2009.09.16	FW14
LL	LL33	119.0	87	76	11	Green	male	2009.09.16	FW14
LL	LL34	171.1	100	88	12	Green	male	2009.09.16	FW14
LL	LL35	130.2	85	76	9	Orchid	male	2009.09.16	FW14
LL	LL36	129.1	88	78	10	Orchid	male	2009.09.16	FW14
LL	LL37	116.4	88	77	11	Orchid	male	2009.09.16	SW14
LL	LL38	147.6	89	79	10	Orchid	male	2009.09.16	SW14
LL	LL39	158.9	96	86	10	Orchid	male	2009.09.16	SW14
LL	LL40	162.1	100	90	10	Orchid	male	2009.09.16	SW14
LL	LL41	146.6	89	78	11	Orchid	male	2009.09.16	SW14
LL	LL42	223.4	104	91	13	Orchid	male	2010.05.13	FW0/SW0
LL	LL43	119.2	84	75	10	Orchid	male	2010.07.10	FW7
LL	LL44	202.4	98	86	12	Orchid	male	2010.07.10	FW7
LL	LL45	220.3	99	86	13	Orchid	male	2010.07.10	SW7
LL	LL46	213.6	97	84	13	Orchid	male	2010.07.10	SW7
LL	LL47	187.8	101	89	12	Orchid	male	2010.07.10	SW2
LL	LL48	182.4	95	83	12	Orchid	male	2010.07.10	FW2
LL	LL49	149.2	88	78	11	Green	male	2010.07.10	FW7

物種	編號	體重(g)	全長(cm)	吻肛長(cm)	尾長(cm)	採集地點	性別	記錄日期	處理組別
LL	LL50	148.8	86.5	76	10.5	Green	male	2010.07.10	SW7
LL	LL51	161.9	97	85	12	Orchid	male	2010.07.16	FW2
LL	LL52	180.9	97	85	12	Orchid	male	2010.07.16	FW2
LL	LL53	161.9	96	83	13	Orchid	male	2010.07.16	SW2
LL	LL54	175.5	97	85	13	Orchid	male	2010.07.16	SW2
LL	LL55	134.7	99	89	13	Orchid	male	2010.08.20	SW2
LL	LL56	151.8	88	78	13	Orchid	male	2010.08.20	SW2
LL	LL57	146.1	86	76	13	Orchid	male	2010.08.20	FW2
LL	LL58	165.5	92	83	13	Orchid	male	2010.08.20	FW2
LL	LL59	171.9	94	83	13	Orchid	male	2010.08.20	FW0/SW0
LL	LL60	193.6	95	85	13	Orchid	male	2010.08.20	FW0/SW0
LC	LC15	182.8	80	70	10	Orchid	male	2009.08.22	SW7
LC	LC16	133.9	79	70	9	Orchid	male	2009.08.22	SW7
LC	LC17	283.6	90	80	10	Orchid	male	2009.08.22	FW7
LC	LC18	121.2	80	70	10	Orchid	male	2009.08.22	FW7
LC	LC19	164.6	86	76	10	Orchid	male	2009.09.16	FW7
LC	LC20	198.2	90	80	10	Orchid	male	2009.09.16	SW7
LC	LC21	219.0	90	80	10	Green	male	2010.04.23	SW7
LC	LC22	118.0	79	69	10	Orchid	male	2010.04.23	SW7
LC	LC23	185.0	88	78	10	Orchid	male	2010.04.23	FW7

物種	編號	體重(g)	全長(cm)	吻肛長(cm)	尾長(cm)	採集地點	性別	記錄日期	處理組別
LC	LC24	320.7	106	92	14	Green	male	2010.05.28	FW0/SW0
LC	LC25	188.0	90	77	13	Green	male	2010.05.28	FW0/SW0
LC	LC26	188.0	85	72	13	Green	male	2010.05.28	FW0/SW0
LC	LC27	180.3	87	76	11	Green	male	2010.05.28	FW2
LC	LC28	289.8	101	88	13	Orchid	male	2010.05.28	FW0/SW0
LC	LC29	208.7	96	84	13	Orchid	male	2010.05.28	FW2
LC	LC30	155.7	84	73	11	Orchid	male	2010.05.28	SW2
LC	LC31	206.2	94	82	13	Orchid	male	2010.05.28	SW2
LC	LC32	226.9	93	81	12	Orchid	male	2010.05.28	FW7
LC	LC33	164.3	85	74	11	Orchid	male	2010.05.28	FW7
LC	LC34	204.2	95	82	13	Orchid	male	2010.07.10	FW0/SW0
LC	LC35	193.5	99	86	13	Orchid	male	2010.07.10	FW2
LC	LC36	166.2	95	81	14	Orchid	male	2010.07.10	FW2
LC	LC37	208.2	92	80	12	Orchid	male	2010.07.10	FW2
LC	LC38	161.4	90	78	12	Orchid	male	2010.07.10	SW2
LC	LC39	143.2	82	72	11	Orchid	male	2010.07.10	SW2
LC	LC40	169.1	89	77	12	Orchid	male	2010.07.10	SW2
LC	LC41	170.2	89	77	12	Orchid	male	2010.07.10	FW7
LC	LC42	222.3	99	85	14	Orchid	male	2010.07.10	SW7
LC	LC43	227.8	94	82	12	Green	male	2010.07.10	FW7

物種	編號	體重(g)	全長(cm)	吻肛長(cm)	尾長(cm)	採集地點	性別	記錄日期	處理組別
LC	LC44	203.7	97	84	13	Green	male	2010.07.10	SW7

註： 1. 物種表示： LS：闊帶青斑海蛇、LL：黑唇青斑海蛇、LC：黃唇青斑海蛇

2. 採集地點：Orchid：蘭嶼、 Green：綠島

3. 處理組別：FW：淡水馴養、SW：海水馴養；數字為轉移後馴養天數