

Behaviour to avoid saltwater sites during spawning in *Buergeria japonica* (Hallowell, 1861)

Takashi Haramura¹

Abstract. The use of spawning sites by female amphibians can strongly influence the rate of egg survival. Coastal areas are risky environments for amphibian breeding because the rate of egg survival falls drastically with increased water salinity. *Buergeria japonica* is an anuran species that breeds in coastal environments but avoids spawning in sites with increased salinity. The aim of this study is to understand the behavioural sequence of how this frog avoids saltwater sites. To address this question, I recorded spawning behaviour in a laboratory experiment. Although amplexant pairs randomly visited and entered fresh and saltwater sites, they rapidly left saltwater sites after entering the water. The amount of time spent in saltwater sites prior to exiting the water declined with increasing water salinity. The selection of a spawning site was determined by gravid females only because each male was clasping onto the back of the female and did not contribute to the locomotion of amplexant pairs. This result suggests that female *B. japonica* are able to quickly determine the salinity level of potential breeding sites. Together, all adaptive responses allow this species to persist in a coastal environment, in which salinity of breeding sites varies greatly.

Keywords. amphibian, amplexant pair, anuran, coastal area, salinity

Introduction

Oviposition sites directly reflect resource quantity and quality, level of predation, competition and the abiotic environment experienced by offspring (Resetarits and Wilbur, 1989; Smith et al., 2000; Murphy, 2003). Therefore, oviposition site use by females strongly affects egg survival rate, hatching success and offspring performance (Resetarits and Wilbur, 1989; Spieler and Linsenmair, 1997; Rieger et al., 2004). Female frogs use abiotic and biotic factors as cues for egg-laying (Seale, 1982; Huk and Kühne, 1999; Wood and Bjorndal, 2000). Especially, when factors that directly influence egg or offspring mortality exist in their habitat, ovipositing females use breeding sites based on these factors (Resetarits and Wilbur, 1989; Kats and Sih, 1992; Laurila and Aho, 1997; Rieger et al., 2004). To improve the chance of reproductive success, females may evaluate the suitability of potential oviposition sites prior to laying through a process of adaptive oviposition site use (Bernardo, 1996; Buxton and Sperry, 2016).

Chemical cues are one of factors used by amphibians to find suitable oviposition sites. Predator avoidance via oviposition site use has been reported for several amphibian species (Resetarits and Wilbur, 1989; Kats and Sih, 1992; Hopey and Petranka, 1994; Spieler and Linsenmair, 1997; Binckley and Resetarits, 2002), and chemical cues of predators as well as intra- or interspecific chemical cues affect oviposition site use (Orizaola and Braña, 2003). Similarly, sites with higher salinity should also be avoided because amphibian egg survivorship decreases dramatically with slight increases in salinity (Gordon and Tucker, 1965, 1968; Beebee, 1985; Uchiyama et al., 1990). *Buergeria japonica* (Hallowell, 1861) is a ground-dwelling rhacophorid frog that occurs in Taiwan and on most islands of the Ryukyu Archipelago of Japan. This frog is known to inhabit coastal areas (Maeda and Matsui, 1989). The sites chosen for oviposition are likely critical for offspring survival in such environments where organisms are subject to complex interactions between marine and terrestrial influences. In brackish or salt water, dehydration occurs through salt gain, and most organisms must regulate the osmolality of body fluids to survive such conditions (Brandley, 2009). Eggs are also influenced by salt water, so females likely assess salinity to determine the quality of spawning sites. Female *B. japonica* can distinguish salinity levels of spawning sites when laying eggs and avoid high salinity sites that are characterised by increased egg mortality (Haramura,

¹ Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan; and Department of Environmental Sciences, Rakuno Gakuen University, Ebetsu, Hokkaido, 069-8501 Japan. E-mail: t-haramura@rakuno.ac.jp

2008). However, Haramura (2008) only focused on spawning data and did not clarify the sequence of spawning behaviour whereby female *B. japonica* avoid high salinity sites. Here, I present the sequence of spawning behaviour of *B. japonica* recorded by video camera during experiment of Haramura (2008). This data allows us to interpret how female *B. japonica* distinguish salinity differences and use appropriate (freshwater) spawning sites.

Materials and Methods

Buergeria japonica is a small frog (2.5–3.7 cm in snout-vent length [SVL]), that occurs in various habitats, from coastal lowlands to forests in mountain areas. Breeding occurs from April to September in slow flowing streams and small pools, such as ditches and puddles (Maeda and Matsui, 1989). I conducted a laboratory experiment at the Yona Research Station, University of the Ryukyus, Okinawa Island, Japan (26.8199°N, 128.3141°E) from May to September 2003 and May to July 2004. I captured 11 amplexant pairs in the field and brought them to the laboratory, each pair being kept separately in a plastic cup. A plastic box (90 cm x 60 cm x 40 cm) was used as the experimental container, in which two dishes (14 cm diameter, 3 cm height) were placed in each side (four dishes were present, in total). Two dishes were filled with aged tap water (freshwater sites), and another two dishes were filled with tap water containing a fixed amount of NaCl (saltwater sites). In each trial, the same salinity level was prepared for the two saltwater dishes. Dishes of fresh and salt water were placed so as to position the same salinity on the diagonal opposite each other. In

control trials (salinity level of four dishes was 0‰) eggs laid in the diagonally opposite dishes were used for the freshwater site or superficial saltwater site. The five salinity levels ranging from 0‰ to 30‰ were tested using 11 amplexant pairs (0‰ [$n = 4$], 3‰ [$n = 1$], 4‰ [$n = 1$], 10‰ [$n = 2$], 30‰ [$n = 3$]). For each trial, an amplexant pair was placed in the experimental container. During trials, frogs were exposed to natural light and temperature, and spawning behaviour was recorded by video camera (Sony, video Hi8 XR) from above. I started each experiment between 00:30 h and 02:30 h and ran it until female frogs laid eggs or amplexant pairs naturally ceased to be amplexant. Further information of the experiment is given in Haramura (2008). I viewed the video recordings and counted the number of visitations to each dish (fresh or saltwater sites) for each pair and calculated the time each pair spent in fresh and salt water. I compared the number of visiting amplexant pairs between fresh and saltwater sites using Pearson's chi-square test and then compared the time spent in water between fresh and saltwater sites using Mann-Whitney *U*-test. I also used regression analysis to compare the time spent in water (fresh or salt water). I performed statistical analyses using JMP 12.2.0 (SAS Institute Inc., 2015) with all significance level tested at $p = 0.05$ (two-tailed).

Results

In seven of the 11 trials with amplexant pairs, females successfully laid eggs in water. For the remaining four trials, females did not deposit any eggs or laid eggs outside of the water dishes (Table 1). In the present study, I analysed the seven successful outlets.

Table 1. Spawning behaviour of amplexant pairs (ID) of *Buergeria japonica* as recorded by video camera data. The number of pairs visiting fresh or saltwater sites, total time spent in fresh or saltwater sites and total time spent in both fresh and saltwater sites (s: seconds). Total record time is the time from start to finish by video camera recording, or to the time the amplexant pair naturally separated.

Pair ID	Salinity (%)	Body mass (g)		Number of freshwater sites visited	Number of saltwater sites visited	Total time in freshwater (s)	Total time in saltwater (s)	Total time staying in water (s)	Total record time (s)
		Male	Female						
1	0	1.9	5.6	11	15	977	2055	3032	14754
2	0	2.0	4.1	4	10	771	793	1564	9758
3	3	1.8	5.9	13	20	1095	422	1517	12145
4	4	1.7	3.9	4	5	1594	208	1802	7960
5	10	1.9	5.5	7	4	5189	91	5277	22627
6	30	2.1	4.2	10	4	1364	20	1384	10040
7	30	2.0	5.1	3	2	927	15	942	10551

The number of amplexant pairs visiting fresh or salt water did not differ significantly (Pearson's chi-square test, $\chi^2 = 7.84$, $p > 0.05$). Therefore, amplexant pairs were randomly visiting fresh and saltwater sites for spawning. However, for the ratio of time staying in water, total time in salt water decreased as salinity level increased (fitting a polynomial, $R^2 = 0.71$, $F = 12.12$, $p < 0.05$, Fig. 1). The time spent in water for each visiting event was different between fresh and salt water, with time in water being lower in saltwater sites (Mann-Whitney U-test, $Z = 3.80$, $p < 0.0001$, Fig. 2). Additionally, in salt water, although the time spent in water for each visiting event decreased as salinity level increased (fitting a polynomial, $R^2 = 0.22$, $F = 16.81$, $p < 0.0001$, Fig. 3A), the time spent in fresh water for each visiting event was not different in each salinity level (fitting a polynomial, $R^2 = 0.02$, $F = 0.97$, $p = 0.33$, Fig. 3B).

Discussion

In the present experiment amplexant pairs of *B. japonica* randomly visited fresh and saltwater sites, but spent less time in salt water than fresh water. Also, as salinity level increased, the time spent in salt water for each visiting event decreased. This means that *B. japonica* can rapidly distinguish the salinity level of water in a potential breeding site and quickly avoid

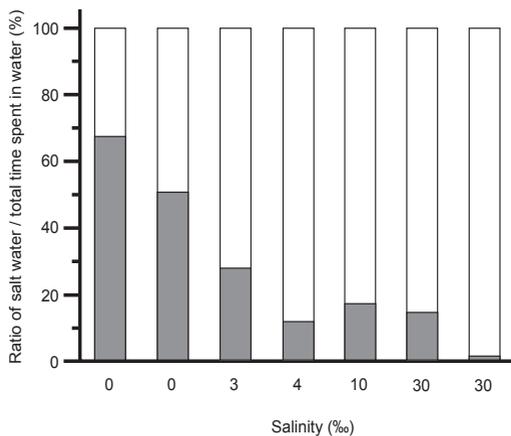


Figure 1. The ratio of time spent in salt water for seven amplexant pairs of *B. japonica*. The percentage of salt water is the time spent in saltwater sites divided by the total time in water (both fresh and saltwater sites). The X axis is salinity levels, and the Y axis is percentage of time spent in fresh and saltwater sites. White colour is time spent in fresh water and grey colour is time spent in salt water.

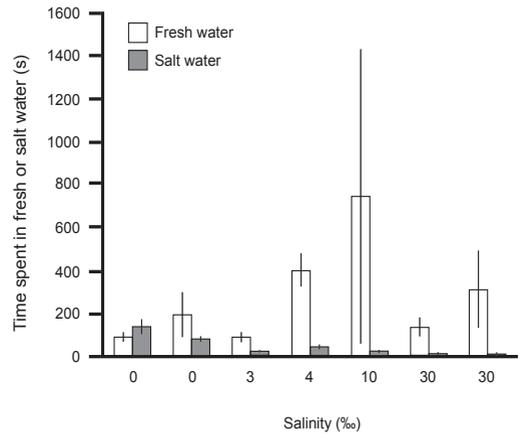


Figure 2. Mean values (\pm SE) of the time spent in fresh and saltwater sites for each salinity level. Time is shown as seconds.

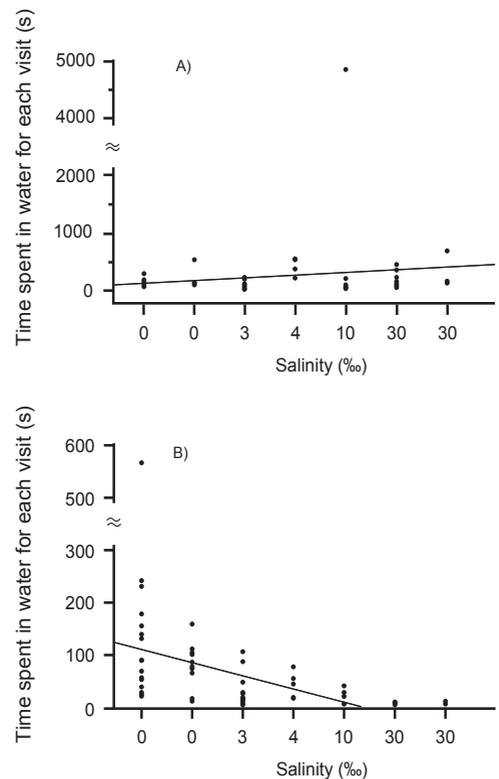


Figure 3. The time spent in water for each visiting event to A) freshwater, and B) saltwater for each salinity level. Time is shown as seconds.

saltwater sites. This avoidance was due to the female assessing the potential of spawning sites regarding salinity, because the male was clasping the back of the female and did not provide any input into movement.

Anurans have the ability to use oviposition sites based on various chemical cues, such as water pH and presence of predators (Spieler and Linsenmair, 1997; Fegraus and March, 2000; Oliveira and Navas, 2004). Several frog species avoid pools with conspecific tadpoles (Crump, 1991; Halloy and Fiaño, 2000; Resetarits and Wilbur, 1991; Spieler and Linsenmair, 1997), and this is likely to be under strong selection pressure (Rudolf and Rödel, 2005). When conspecific predaceous tadpoles are present in a waterbody, adults can assess the presence of such tadpoles using chemical cues released by the tadpoles themselves or injured conspecifics (Spieler and Linsenmair, 1997). Similarly, female newts (*Triturus marmoratus*) also use chemical cues of predators to avoid oviposition in potentially risky situation (Orizaola and Braña, 2003). Jointly, these results suggest that breeding amphibian females have evolved the ability to detection and avoid chemical cues associated with high-mortality risk for eggs and/or larvae. High salinity is one of these mortality factors (Duellman and Trueb, 1986) so that breeding females are predicted to use oviposition sites based on the assessment of salinity (Viertel, 1999; Haramura, 2008).

The mechanisms by which breeding amphibians assess salinity vary among species. Lorrain-Soligon et al. (2022) showed that *Pelophyax* spp. assess salinity without direct contact with the water. Amphibians have been shown to detect polarised light (Taylor and Auburn, 1978; Phillips et al., 2001) and use light polarisation patterns for orientation (Taylor and Auburn, 1978). However, while some species possess the ability for such visual assessment of salinity, most species require contact between water and chemosensory organs to determine the salinity of waterbodies (Sugarman et al., 1983; Martín et al., 2021). Here, *B. japonica* showed an inability to visually assess water salinity because females randomly approached and entered fresh and saltwater sites. I therefore suggest that, as shown in many other amphibian species, this species recognises salinity level through the permeable skin, most likely the ventral skin.

The permeable skin of amphibians makes them particularly susceptible to salinity (Hillyard, 1999; Wake and Koo, 2018). For example, Wood Frogs (*Lithobates sylvaticus*) can distinguish between soils that differ in salinity and avoid salts in terrestrial soils

(Vegso et al., 2022). The epithelial Na⁺ transport acts in a chemosensory function (Hoff and Hillyard, 1993; Nagai et al., 1999). In the present study, chemosensory functions would likely be a predominant cue for distinguishing the salinity of experimental breeding sites. Hydroregulatory mechanisms would be particularly important in coastal environments where organisms are subject to a mosaic of marine and terrestrial influences. In salt water, dehydration occurs through salt gain and water loss, and most organisms have to regulate the osmolality of their body fluids in order to survive (Bradley, 2009). To date, various pieces of evidence suggest that amphibians are able to avoid saltwater sites for spawning. Further research is required to understand whether breeding female *B. japonica* from inland (mountain) populations have the ability to assess and avoid saltwater sites. Namely, *Buergeria japonica* from the coastal population have higher Na⁺/K⁺-ATPase activity than in the inland population (Haramura et al., 2021). Also, the larvae of other amphibian species in coastal environments have an increased abundance of aquaporins and ion pumps (e.g., Na⁺/K⁺-ATPase) in the gills (Bernobò et al., 2013; Wu et al., 2014). Na⁺/K⁺-ATPase is an important protein for osmoregulation in high salinity, and increased expression of Na⁺/K⁺-ATPase under both hypo- and hyper-osmotic stresses is regarded as an indicator of osmoregulation (Hwang and Lee, 2007; Hwang et al., 2011). Further research on the evolution of spawning site use based on assessment of water salinity, both within and among species, would improve our understanding of the ecology and geographic distribution of amphibians.

Acknowledgments. I thank A. Mori for his valuable comments on my research. Yona Research Station, University of the Ryukyu, provided facilities during my research. H. Ota provided the instrument for measuring salinity. I also thank M. Crossland for proofreading this manuscript for English grammar.

References

- Beebee, T.J.C. (1985): Salt tolerances of natterjack toad (*Bufo calamita*) eggs and larvae from coastal and inland populations in Britain. *Herpetological Journal* **1**: 14–16.
- Bernardo, J. (1996): Maternal effects in animal ecology. *American Zoologist* **36**: 83–105.
- Bernobò, I., Bonacci, F., Coscarelli, M., Tripepi, M., Bruneli, E. (2013): Effects of salinity stress on *Bufo balearicus* and *Bufo* tadpoles: tolerance, morphological gill alterations and Na⁺/K⁺-ATPase localization. *Aquatic Toxicology* **132**: 119–133.
- Binckley, C.A., Resetarits, W.J., Jr. (2002): Reproductive decisions under the threat of predation: squirrel treefrog (*Hyla squirella*) responses to banded sunfish (*Enneacanthus obesus*). *Oecologia*

- 130:** 157–161.
- Bradley, T.J. (2009): Animal Osmoregulation. New York, USA, Oxford University Press.
- Buxton, V.L., Sperry, J.H. (2016): Reproductive decisions in anurans: A review of how predation and competition affects the deposition of eggs and tadpoles. *BioScience* **67:** 26–38.
- Crump, M.L. (1991): Choice of oviposition site and egg load assessment by a treefrog. *Herpetologica* **47:** 308–315.
- Duellman, W.E., Trueb, L. (1986): Biology of Amphibians. New York, USA, McGraw-Hill Book Co.
- Fegraus, E.H., Marsh, D.M. (2000): Are newer ponds better? Pond chemistry, oviposition site selection, and tadpole performance in the tungara frog, *Physalaemus pustulosus*. *Journal of Herpetology* **34:** 455–459.
- Gordon, M.S., Tucker, V.A. (1965): Osmotic regulation in the tadpoles of the crab-eating frog (*Rana cancrivora*). *Journal of Experimental Biology* **42:** 437–445.
- Gordon, M.S., Tucker, V.A. (1968): Further observations on the physiology of salinity adaptation in the crab-eating frog (*Rana cancrivora*). *Journal of Experimental Biology* **49:** 185–193.
- Hallo, M., Fiaño, J.M. (2000): Oviposition site selection in *Pleurodema borellii* (Anura: Leptodactylidae) may be influenced by tadpole presence. *Copeia* **2000:** 606–609.
- Haramura, T. (2008): Experimental test of spawning site selection by *Buergeria japonica* (Anura: Rhacophoridae) in response to salinity level. *Copeia* **2008:** 64–67.
- Haramura, T., Mohammadi, S., Savitzky, A.H. (2021): Relative differences in Na⁺/K⁺-ATPase activity between mountain and coastal populations of the Japanese frog, *Buergeria japonica*. *Journal of Herpetology* **55:** 338–341.
- Hillyard, S.D. (1999): Behavioral, molecular and integrative mechanisms of amphibian osmoregulation. *Journal of Experimental Zoology* **283:** 662–674.
- Hoff, K., Hillyard, S.D. (1993): Toads taste sodium with their skin: sensory function in a transporting epithelium. *Journal of Experimental Biology* **183:** 347–351.
- Hopey, M.E., Petranka, J.W. (1994): Restriction of wood frog to fish-free habitats: how important is adult choice? *Copeia* **1994:** 1023–1025.
- Huk, T., Kühne, B. (1999): Substrate selection by *Carabus clatratus* (Coleoptera, Carabidae) and its consequences for offspring development. *Oecologia* **121:** 348–354.
- Hwang, P.P., Lee, T.H. (2007): New insights into fish ion regulation and mitochondrion-rich cells. *Comparative Biochemistry and Physiology A* **148:** 479–497.
- Hwang, P.P., Lee, T.H., Lin, Y.H. (2011): Ion regulation in fish gills: recent progress in the cellular and molecular mechanisms. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **301:** R28–R47.
- Kats, L., Sih, A. (1992): Oviposition site selection and avoidance of fish by streamside salamanders (*Ambystoma barbouri*). *Copeia* **1992:** 468–473.
- Laurila, A., Aho, T. (1997): Do female common frogs choose their breeding habitats to avoid predation in tadpoles? *Oikos* **78:** 585–591.
- Lorrain-Soligon, L., Robin, F., Brischoux, F. (2022): Hydric status influences salinity-dependent water selection in frogs from coastal wetland. *Physiology and Behavior* **249:** 113775.
- Martín, J., Ibáñez, A., Garrido, M., Raya-García, E., López, P. (2021): Chemical cues may allow a fossorial amphibiaenian reptile to avoid extremely salinity soils when selecting microhabitats. *Journal of Arid Environments* **188:** 104452.
- Maeda, N., Matsui, M. (1989): Frogs and toads of Japan. Tokyo, Japan, Bun-Ichi Sogo Shuppan Co. Ltd. [in Japanese]
- Murphy, P.J. (2003): Does reproductive site choice in a Neotropical frog mirror variable risks facing offspring? *Environmental Monographs* **73:** 45–67.
- Nagai, T., Koyama, H., Hoff, K.V.S., Hillyard, D. (1999): Desert toads discriminate salt taste with chemosensory function of the ventral skin. *Journal of Comparative Neurology* **408:** 125–136.
- Oliveira, F.B., Navas, C.A. (2004): Plant selection and seasonal patterns of vocal activity in two populations of the bromeligen treefrog *Scinax perpusillus* (Anura, Hylidae). *Journal of Herpetology* **38:** 331–339.
- Orizaola, G., Braña, F. (2003): Do predator chemical cues affect oviposition site selection in newts? *Herpetological Journal* **13:** 189–193.
- Phillips, J.B., Deuschlander, M.E., Freake, M.J., Borland, S.C. (2001): The role of extraocular photoreceptors in newt magnetic compass orientation: parallels between light-dependent magnetoreception and polarized light detection in vertebrates. *Journal of Experimental Biology* **204:** 2543–2552.
- Resetarits, W.J., Jr., Wilbur, H.M. (1989): Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors. *Ecology* **70:** 220–228.
- Resetarits, W.J., Jr., Wilbur, H.M. (1991): Calling site choice by *Hyla chrysoscelis*: Effect of predators, competitors, and oviposition sites. *Ecology* **72:** 778–786.
- Rieger, J.F., Binckley, C.A., Resetarits, W.J., Jr. (2004): Larval performance and oviposition site preference along a predation gradient. *Ecology* **85:** 2094–2099.
- Rudolf, V.H., Rödel, M.-O. (2005): Oviposition site selection in a complex and variable environment: The role of habitat quality and conspecific cues. *Oecologia* **142:** 316–325.
- Seale, D.B. (1982): Physical factors influencing oviposition by the Woodfrog, *Rana sylvatica*, in Pennsylvania. *Copeia* **1982:** 627–635.
- Smith, C.J., Reynolds, J.D., Sutherland, W.J. (2000): Population consequences of reproductive decisions. *Proceedings of the Royal Society of London Series B: Biological Sciences* **267:** 1327–1335.
- Spieler, M., Linsenmair, K.E. (1997): Choice of optimal oviposition sites by *Hoplobatrachus occipitalis* (Anura: Ranidae) in an unpredictable and patchy environment. *Oecologia* **109:** 184–199.
- Sugarman, P.C., Pearson, W.H., Woodruff, D.L. (1983): Salinity detection and associated behavior in the Dungeness crab, *Cancer magister*. *Estuaries* **6:** 380–386.
- Taylor, D.H., Auburn, J.S. (1978): Orientation of amphibians by linearly polarized light. In: *Animal Migration, Navigation, and Homing*, p. 334–346. Schmidt-Koenig, K., Keeton, W.T., Eds., Heidelberg, Berlin, Germany, Springer-Verlag.
- Uchiyama, M., Murakami, T., Yoshizawa, H. (1990): Notes on the development of crab-eating frog, *Rana cancrivora*. *Zoological Science* **7:** 73–78.
- Vegso, Z.T., Kalonia, A.A., Stevens, S., Rittenhouse, T.A. (2022):

- Salinity conditions during the larval life stage affect terrestrial habitat choice in juvenile wood frogs (*Lithobates sylvaticus*). *Journal of Herpetology* **56**: 60–66.
- Viertel, B. (1999): Salt tolerance of *Rana temporaria*: spawning site selection and survival during embryonic development (Amphibia, Anura). *Amphibia-Reptilia* **20**: 161–171.
- Wake, D.B., Koo, M.S. (2018): Amphibians. *Current Biology* **28**: R1237–1241.
- Wood, D.W., Bjorndal, K.A. (2000): Relation of temperature, moisture, salinity, and slope to nest site selection in Loggerhead Sea Turtles. *Copeia* **2000**: 119–128.
- Wu, C.S., Yang, W.K., Lee, T.H., Gomez-Mestre, I., Kam, Y.C. (2014): Salinity acclimation enhances salinity tolerance in tadpoles living in brackish water through increased Na⁺, K⁺-ATPase expression. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **321**: 57–64.
- Zhang, X., Stramski, D., Reynolds, R.A., Blocker, E.R. (2019): Light scattering by pure water and seawater: the depolarization ratio and its variation with salinity. *Applied Optics* **58**: 991–1004.