Integrated Research of Coastal Development Effects on Tachypleus tridentatus and the Estimation of Its Suitable Spawning Habitat in Tsuyazaki Cove, Japan

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https://hdl.handle.net/2324/7182424

出版情報：Kyushu University, 2023, 博士（工学）, 課程博士
バージョン：
権利関係：

Integrated Research of Coastal Development Effects on *Tachypleus tridentatus* and the Estimation of Its Suitable Spawning Habitat in Tsuyazaki Cove, Japan

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CERTIFICATION

The undersigned hereby certify that they have read and recommended to the Graduate School of Engineering for the acceptance of this dissertation entitled, "**Integrated Research of Coastal Development Effects on *Tachypleus tridentatus* and the Estimation of Its Suitable Spawning Habitat in Tsuyazaki Cove, Japan**" by Shinji Itaya in partial fulfilment of the requirements for the degree of Doctor of Engineering.

March, 2024

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Acknowledgements

I extend my heartfelt appreciation to the following individuals who have played crucial roles in my academic journey:

First and foremost, I sincerely thank my supervisor, Professor Shinichiro Yano, from the Environmental Fluid Dynamics Laboratory at the Graduate School of Engineering, Kyushu University. Professor Yano's kindness in granting me the opportunity to study in his laboratory, along with his unwavering guidance, invaluable support, encouragement, and numerous other forms of assistance throughout this project, has been instrumental in my academic journey. I would also like to express my heartfelt thanks to Dr Akira Tai for his expert advice and generous assistance, including sharing equipment during my fieldwork. I also express my gratitude to the examination committees, Professor Masaru Yamashiro and Professor Takahiro Kuba, for their thorough review and valuable insights provided for this dissertation.

I sincerely thank Professor Norio Onikura and Assistant Professor Akihiko Koyama from the Fishery Research Laboratory, Department of Bioresource Science, Graduate School of Bioenvironmental Science at Kyushu University. Their generosity in providing access to laboratory facilities and field equipment, as well as their insightful comments, have significantly elevated the quality of my work. I must particularly acknowledge Dr Akihiko Koyama for his invaluable assistance, ranging from GIS-related issues to statistical analysis and his valuable advice on the manuscripts of each published paper.

I also want to express my thanks to Dr Graeme K. Ward from the Australian National University, Dr Mark L. Botton, Professor of Biology at Fordham University, Dr Yumiko Iwasaki from Molloy College & CERCOM, Dr Siddhartha Pati from Natnov Bioscience and Association For Biodiversity Conservation & Research and Dr Koichi Nakama from Tainan University of Technology for their generous support in proofreading, editing, and reviewing my article titled

"Cumulative Modifications of the Endangered Horseshoe Crab *Tachypleus tridentatus* Habitat by Small-Scale Developments at Tsuyazaki Cove in Fukuoka, Japan," which was published in 2022 as part of the book "International Horseshoe Crab Conservation and Research Efforts: 2007-2020." Additionally, I appreciate Dr Kit Yue Kwan from the Beibu Gulf University, China, Dr Bryan R. Nelson from the Universiti Malaysia Terengganu, Malaysia, and N. Ashikin M. Zauki, also from the Universiti Malaysia Terengganu, Malaysia, for sharing their research papers, explaining their findings, and providing valuable comments on my research.

I am immensely grateful to the individuals who contributed to the fieldwork, including Kota, Daichi and Tsubasa Shuuno, Ayumu Sakurada, Ryuto Koshiguchi, Kazuya Maeda, Masaru Ono, Daiki Masuda, Hiroyuki Oyama, Akira Yano, Masashi Imamura, Yurika Umeki, and the numerous students from the Fukuoka Eco Communication College. Their enthusiastic participation in the spawning surveys and horseshoe crab spawning ground measurements was instrumental to the success of this research. I also extend my appreciation to the residents of Tsuyazaki, including the Fukutsu History Society and the local fishing industry, for their cooperation in the interview survey.

I dedicate this dissertation first to the memory of Katsuji Motojima, my "big brother" in environmental consulting, who tragically passed away in a sudden road accident. His encouragement, arrangement of survey equipment, and support during my doctoral studies were invaluable.

Special thanks are reserved for Mari Shuuno and Dr Toshifumi Wada, with whom I began horseshoe crab spawning surveys in Tsuyazaki in 2005. Both of them dedicated themselves wholeheartedly to the study and conservation efforts of the local population. Together, we faced numerous conservation challenges. Sadly, Dr Wada passed away in November 2018. He was a passionate ecologist, conservationist, and thoughtful mentor who proofread the draft of my first paper, published in 2019 and provided me with valuable and sincere feedback. It is with deep respect that I also dedicate this dissertation to his memory.

My heartfelt thanks go to the remarkable individuals whose love and support enabled me to complete this Ph.D. -Dr John A. Harris and the Ward family. Words cannot adequately convey my deep appreciation for Dr John A. Harris, former Head of the School of Environmental Science at the University of Canberra, Australia. He profoundly influenced my appreciation and empathy for all non-human species and the natural world. My journey into the aesthetics of nature conservation began during my student days at the University of Canberra. John stood out for his unique teaching approach, aiming to instil in his students something more profound than just knowledge - a way of perceiving Nature and comprehending its interactions with people and society. John even visited me in Japan during my Ph.D. course, and together, we explored my study site. His insights, including contributions to my Ph.D. thesis content, were invaluable. He meticulously reviewed the draft of my Ph.D. dissertation. I extend my heartfelt gratitude for his unwavering mentorship and friendship. I also wish to extend my heartfelt appreciation to my "Aussie family", the Ward family - Graeme, Sue, Lucy, Buffy, and Lux. They have enriched my life in countless ways, from academic support to daily happiness. The time I spent with them in Canberra holds a special place in my heart.

Influenced by these remarkable individuals, my interest in becoming a scholar began during my student life in Australia. However, my circumstances at the time did not allow me to continue my studies there. Upon returning to Japan, I embarked on a career as an environmental consultant. Yet, my aspiration to pursue a PhD remained constant in my thoughts. This desire persisted, even as I carried out my daily responsibilities. Balancing doctoral studies with conducting regular work presented challenges, and taking that step required great courage. However, it was not my way to leave this aspiration unfulfilled. As I approach the culmination of this lengthy journey, which commenced during my time in Australia, I can finally see the light at the end of the tunnel as I complete this thesis. I am convinced that my research has successfully transformed my comprehension of population ecology into a compelling narrative within conservation biology. It has been a profound and extensive journey, marking a new beginning in my life.

Finally, I also dedicate this dissertation to my family: my mother, Tomika; my sister, Naomi; my brother, Hidetoshi; my niece, Hitomi; and my nephew, Ken. I am deeply grateful for their unwavering support and for always being there as I navigate my life path.

Table of Contents

Chapter 3 Evaluation of the Spawning Site Selection and Nesting Patterns of *Tachypleus tridentatus* in the Modified Spawning Habitat…....................………….……….......... 62

List of Figures

List of Tables

List of Appendices

Abstract

Horseshoe crabs have significant biological and medical importance. They serve as indicators of the ecological health of coastal environments and play a pivotal role in medical testing due to the essential nature of their blood. In Asia, their survival is threatened by the loss of their habitats due to coastal development and the exploitation of horseshoe crabs for economic and medical purposes. Despite these escalating demands, a critical conservation challenge lacks essential baseline data on horseshoe crabs' populations, distribution, and habitat status.

The critically endangered horseshoe crab species, *Tachypleus tridentatus*, occurs in Japan. The species is distributed in northern Kyushu and the Seto Inland Sea, and has experienced a significant population decline, primarily related to habitat alterations from human activities like landfilling and associated coastal development. The spawning behaviour of *T. tridentatus* is closely tied to specific sandy beach environments within inner bays. The physical characteristics of these spawning grounds, as studied in Asian regions, include, for example, nesting zone, nest temperature, water content, and sedimentary properties. There is increasing urgency to investigate the causal relationships between coastal development and the declining *T. tridentatus* populations, as well as to create scientific models to assess these effects on their spawning habitats. However, the previous research lacks a comprehensive analysis linking spawning data and physical parameters to evaluate the impact of coastal development on the *T. tridentatus* spawning habitat. Also, the development of scientific models to elaborate these causal relationships remains largely unexplored. Consequently, despite the alarming threat of global extinction of *T. tridentatus* populations, scientific research has not comprehensively elucidated the underlying factors responsible for this decline.

This research aims to explore these unexplored scientific aspects and offer general conclusions applicable to conserving *T. tridentatus* spawning grounds. Tsuyazaki Cove in Fukuoka,

renowned as a core site for *T. tridentatus* studies in Japan, was chosen for its suitability in achieving these aims. The research objectives include a long-term investigation into the historical impacts of coastal development on spawning grounds, as well as integrating biological data with pertinent physical parameters to estimate these effects on the spawning sites. This comprehensive research study is the first published quantitative evidence of the potential impact of coastal development on *T. tridentatus* spawning habitats and the subsequent population decline.

This dissertation is structured as follows: it begins with an introduction (Chapter 1) followed by four chapters, each published in refereed international journals (Chapters 2, 3, 4, 5). Finally, there is a concluding chapter (Chapter 6) . Chapter 1 introduces the research background, previous studies and their problems, research aims and objectives, proposed methodology and methods, study site description, and overall dissertation structure. Chapter 2 sets the stage for subsequent studies by identifying the need to explore specific elements contributing to the decline. It focused on historical spawning habitat changes through aerial photograph analysis, interviews, and field monitoring of spawning numbers. The findings attributed the decrease of *T. tridentatus* to the cove entrance modification, leading to disrupted sediment transport routes, which caused the degradation of spawning habitats in the area. Chapter 3 narrows its target to the spawning site selection and nesting patterns of *T. tridentatus*, unveiling modified beach shapes attributed to landfills and corresponding changes in nesting behaviours. The study revealed that some eggs were laid in lower intertidal zones, previously considered "unsuitable" for spawning. This shift in egg deposition occurred when the original beach shape had been altered due to coastal development. The study underscored the significance of preserving original beach shapes to uphold the presence of appropriate spawning habitats. In Chapter 4, a field experiment was conducted to test the hypothesis formulated based on the findings in Chapter 3. Specifically, the experiment sought to ascertain the survival rates of eggs deposited within a previously considered "unsuitable" zone. It was achieved by translocating the eggs to various intertidal elevations. The study indicated the critical role of beach elevation in egg survival. The mid intertidal zone was the most suitable, influenced by appropriate air exposure and water content. In contrast, eggs within the low intertidal zone exhibited the least survival rate, suggesting that the area where the original beach morphology underwent significant modifications due to coastal development was judged "unsuitable" for optimal egg development. Chapter 5 leverages the knowledge gained from previous chapters to understand the species' spawning habitat requirements comprehensively and proposes a tangible approach to conservation efforts. The study identifies the specific spawning conditions and areas where *T. tridentatus* can thrive, making it a logical extension of the investigations into spawning site selection (Chapter 3) and egg survival (Chapter 4). Given the absence of scientifically established suitable spawning habitats for *T. tridentatus* in previous studies, in Chapter 5, scientific models based on naturally spawned nest/egg occurrence data and their physical parameters were employed to quantify the extent of remaining suitable spawning habitats in the study site. The remaining suitable spawning areas were also spatially mapped based on the estimated results. The study highlighted that beach elevation was the predominant determinant for estimating spawning habitat suitability. Furthermore, it emphasised that within the study site, the persisting suitable areas measure a mere 476m^2, suggesting that coastal development has engendered the fragmentation of the spawning beaches. Chapter 6 serves as the conclusion and offers recommendations for conserving the local population. Conservation strategies are proposed, including protecting remaining spawning grounds, beach nourishment, restoring sand supply, and implementing legal regulations. Finally, further research, which falls beyond the scope of this dissertation, is recommended.

This systematic approach ensures that each study builds upon the previous one, contributing to a comprehensive understanding of the species' spawning habitat and ultimately culminating in specific strategies for conservation. The published research studies identify the causal links between coastal development and the declining *T. tridentatus* populations in the area, underscoring the importance of habitat conservation and the restoration of suitable spawning sites for this critically endangered species. The insights from these collective studies can be further applied to *T. tridentatus* populations facing global extinction.

Chapter 1 Introduction

1.1 Background

Horseshoe crabs are the only extant Xiphosurans. They are often called "living fossils” because their morphological characteristics have been preserved over millions of years (Akbar John et al., 2018). They are considered to be important marine organisms when discussing biological evolution (Rudkin & Young, 2009). The eggs of horseshoe crabs also serve a crucial ecological function in coastal ecosystems by providing a significant food source for migratory birds (Botton & Shuster, 2003; Botton, 2009). Additionally, horseshoe crab blood possesses a unique natural resource capable of rapidly detecting bacterial endotoxin (Wittenberg, 2021; Liu et al., 2022). Hence, horseshoe crabs are biologically significant and have substantial medical and commercial importance.

The four extant horseshoe crab species are the Atlantic horseshoe crab (*Limulus polyphemus*), the tri-spine horseshoe crab (*Tachypleus tridentatus*), the coastal horseshoe crab (*Tachypleus gigas*), and the mangrove horseshoe crab (*Carcinoscorpius rotundicauda*). The latter three inhabit Asian waters (Sekiguchi, 1999; Akbar John et al., 2018). Horseshoe crabs in Asia are threatened by escalating human impacts such as coastal development, which is regarded as the most severe threat (Akbar John et al., 2018; Laurie et al., 2019; Wang et al., 2020). The necessity to conserve horseshoe crabs in the Asian region has expanded rapidly in recent years because of the increased medical demand for *Tachypleus* amebocyte lysate (TAL) (Akbar John et al., 2018). TAL is extracted from the *T. tridentatus* and *T. gigas* blood and used for testing dialysis-related products in hospitals and biological products that need to be free of endotoxin residues, such as therapeutic vaccines. Thousands of horseshoe crabs are caught annually in China for this purpose alone (Liu et al., 2022). Despite the growing demand and potential threats, many Asian countries lack vital baseline information on horseshoe crabs, including data on their population size, distribution and habitat status. This gap in knowledge underscores the urgent need for concerted efforts to conserve these extraordinary arthropods (Wang et al., 2020).

The only horseshoe crab species found in Japan is *T. tridentatus*. The species also inhabits various South and East Asian countries and regions, including Indonesia, Malaysia, Vietnam, the Philippines, China, Hong Kong, Taiwan, and Japan (Sekiguchi, 1989; Sekiguchi, 1999; Vestbo et al., 2018). Japan marks the northern boundary of the distribution range for *T. tridentatus* (Laurie et al., 2019). Throughout its life cycle, the species relies on various habitat types in coastal areas. For instance, adult *T. tridentatus* nest on sandy beaches found in calm semi-enclosed bays, while juveniles inhabit sandy mudflats, and sub-adults seek refuge in seaweed beds (Sekiguchi, 1989; Seino et al., 1998a; Sekiguchi, 1999). *T. tridentatus* is considered an indicator species for monitoring the health and ecological condition of coastal environments because it lives in such diverse habitats (Seino & Uda, 2002; Hsieh & Chen, 2009).

The spawning of *T. tridentatus* takes place in summer from late June to August in Japan. During this period, females lay their eggs on the shallow slopes of sandy beaches near the high tide line. Subsequently, the hatched larvae usually disperse to nearby sandy mudflats in the following autumn. However, some larvae overwinter within the same nests and later relocate to adjacent sandy mudflats during the following spring (Sekiguchi, 1999; Maeda et al., 2000; Wada et al., 2010).

Typical of arthropods, *T. tridentatus* moults as they transition from the larval phase to the sexually mature. During each moult, its body size increases by approximately 1.28 times. To achieve successful moulting, males require at least 13 moulting stages, while females require 14 stages. This moulting process takes approximately 14 years for males and 15 years for females from the time they hatch in a controlled captive breeding environment (Sekiguchi, 1999).

The species was once widely distributed in northern Kyushu and the Seto Inland Sea. However, it now has endangered status due to a significant decline in populations, primarily attributed to habitat alterations caused by human activities, such as landfilling (Table 1.1) (Sekiguchi, 1989; Seino et al., 2000a; Seino et al., 2001b; Seino et al., 2002). The adult *T. tridentatus* populations have been dramatically reduced in Japan, with a 90% decline in adults occurring after World War II (Nishi, 1975). The total adult population was estimated to be several hundred thousand individuals in the early 1990s but has now significantly decreased to less than 10,000 individuals (M.E., 2014). Only small and fragmented spawning habitats remain in Okayama, Hiroshima, Yamaguchi, Oita, Fukuoka, Saga, and Nagasaki prefectures (Ohtsuka et al., 2017) (Table 1.1 and Figure 1.1). Japan is one of the countries where the most devastating habitat destruction of *T. tridentatus* has occurred (Sekiguchi, 1989; Botton, 2001). Consequently, in Japan, the species is listed as Critically Endangered (Ito, 2014) compared to its listing as Endangered on the Red List of the International Union for Conservation of Nature (Laurie et al., 2019).

With the growing recognition of the significance of coastal conservation, traditional civil engineering and commercial constructions are being reassessed. Alternative development approaches are being sought to ensure harmonious coexistence with *T. tridentatus* (Seino et al., 2000a). Consequently, comprehensive research and efficient conservation measures become paramount, with ecological conservation and the restoration of spawning habitats emerging as critical priorities for the species' survival (Hsieh & Chen, 2009; Wada et al., 2010).

Recently, acoustic telemetry into the annual migration of adult *T. tridentatus* demonstrated that more than 60% of the tagged individuals exhibited robust fidelity to their original habitats (Wada et al., 2016). Genetic analysis of *T. tridentatus* in Japan has disclosed the existence of at least six conservation management units (the Seto Inland Sea, Fukuoka, Kafuri, Imari, Nagasaki, and Iki units) (Figure 1.1), indicating the importance of implementing specific regional conservation measures (Nishida & Koike, 2009). As supported by these telemetry and genetic studies showing limited movement of populations between specific regions, conducting research and devising practical conservation strategies tailored to each population is considered vital.

Table 1.1 Description of the past and current population status of *T. tridentatus* in Japan (sources: Nishii 1975; Sekiguchi, 1989; Seino et al., 1998a; Sekiguchi, 1999; Hino, 2008; Takeishi, 2016).

Region	Prefecture	Population Status in the Past	Current Status
Honshu Island	Osaka	The species is believed to have been in the area until the 1960s.	Extinct
	Hyogo	The species is believed to have inhabited the area in the 1930s.	Extinct
	Okayama	In the 1920s, Kasaoka was renowned as one of the largest spawning sites for *T. tridentatus* . The area was even designated as a natural monument in 1928 due to its significance. During the 1960s, a substantial portion of the population, comprising at least 10,000 adults, vanished due to extensive landfills.	Since the 1970s, the species' artificial breeding and reintroduction programmes have been conducted. Successful reproduction in natural environments has been confirmed since 2009, but only in small numbers.
	Hiroshima	Previously abundant along the coastal area, the population experienced a decline during the 1960s.	Since 2000, breeding of the species has been confirmed only in Takehara City and Etajima Bay.
	Yamaguchi	The population was once abundant until the 1960s to 1970s.	Breeding has been confirmed since 2000 only in Hirao Bay, Yamaguchi Bay, Chidori Beach, and Maeba River.
Shikoku Island	Ehime	The species was once abundant until the 1960s.	Breeding was confirmed only in Yoshiumi Bay in 1995 and 1996. Juveniles were released at Kawarazu Beach, but breeding has not been confirmed.
	Kagawa	The species was once abundant until the 1960s.	A few individuals survive on the Shiaku Islands.
	Tokushima	Once abundant.	Extinct
Kyushu Island	Oita	The species was once abundant, but the population has declined since 1981.	Breeding has been confirmed only in Beppu (Morie) Bay and Nakatsu City (Yamakuni River).
	Fukuoka	In the 1930s, the species was abundant along the entire coastal area of Karatsu Bay and Hakata Bay. Additionally, in 1983, it was found to inhabit Kitakyushu City, specifically the Sone tidal flat and Kitaku areas.	Breeding has been confirmed only in Imazu, Kafuri, Sone, and Tsuyazaki.
	Saga	Until the 1980s, the species was abundant and inhabited the entire coastal area of Karatsu Bay and Imari Bay.	In the 1990s, the Imari River mouth was considered the largest breeding site, but the population has declined.
	Nagasaki	Until the 1990s, the horseshoe crab population was abundant along the coastal areas of North Matsuura, Kujyukushima, Sasebo, Ohmura, and West Sonogi. It was also once abundant in Tsushima and Iki Islands.	Breeding has been confirmed only in Kujyukushima, Saikai, and Iki.

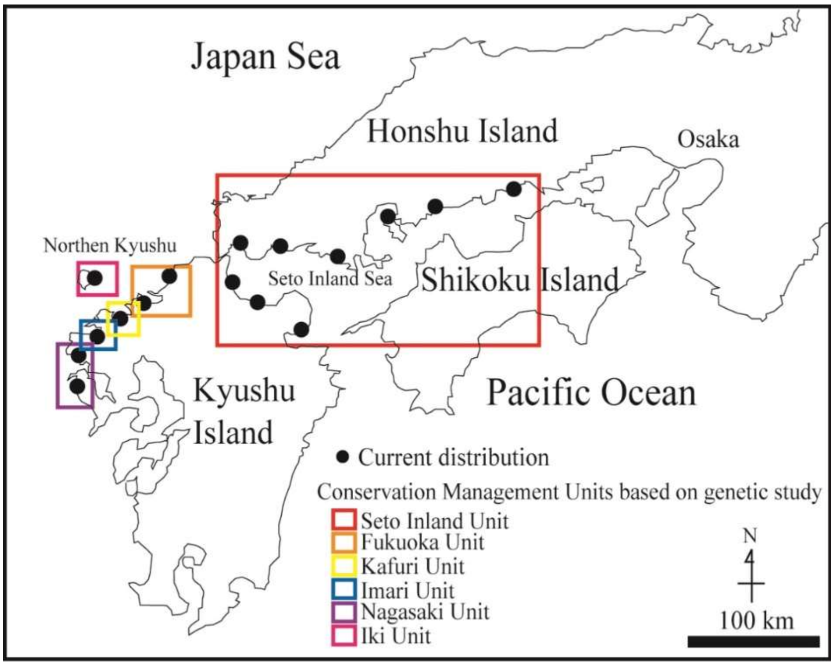

Figure 1.1 Current distribution (Sekiguchi, 1989; Sekiguchi, 1999; Hino, 2008; Takeishi, 2016) and conservation management units based on genetic studies (Nishida & Koike, 2009) of *T. tridentatus* in Japan.

1.2 Review of Previous Studies and Problem Statement

Effective monitoring of population dynamics and thorough investigation into the underlying factors responsible for the targeted species decline are imperative research priorities in conservation ecology. These critical endeavours are necessary to safeguard and prevent rare species from reaching the brink of extinction (Miyashita & Fujita, 1996). However, baseline information on horseshoe crab populations, distribution, and habitat status is insufficient in many Asian countries. Furthermore, apart from Japan, most of the studies have focused on juveniles, with limited research being conducted on spawning ecology and the conservation of spawning grounds (Wang et al., 2020).

T. tridentatus nest on sandy beaches with calm wave conditions within inner bays (Sekiguchi, 1999). These specific beach locations are favoured as spawning grounds for *T. tridentatus* because there are calm wave conditions and gentle tidal currents, which assist in the accumulation of suitable sediments and maintenance of the relative stability of the beach shapes (Seino et al.,1998a; Seino et al.,2000b; Seino et al.,2001b). The physical characteristics of the spawning grounds of *T. tridentatus* that have been studied in Asian regions are as follows.

It has been confirmed that there is a higher frequency of nesting in the intertidal zone between the mean higher high water (MHHW) and the mean higher water neaps (MHWN) (Wakamiya, 1989; Sekiguchi, 1999; Mohamad et al., 2019). This particular spawning range is intricately linked to the morphology of the beach and the local tidal range. Nesting elevations have been reported ranging from T.P. +0.1 to T.P. +1.3 m, with beach slopes ranging from 3.8 to 7.1 degrees (Seino et al., 1998a; Seino et al., 2000a; Ohtsubo et al., 2005; Hsieh & Chen, 2015).

Additional characteristics associated with beach morphology and the local tidal range have been recorded, including nest temperatures ranging from 22.0 to 27.6°C, water content varying from 3.7 to 25.0%, and the nests' duration of air exposure, which spans from 12.0 to 17.0 hours per day (Sekiguchi, 1999; Maeda et al., 2000; Chen et al., 2004; Ohtsubo et al., 2005; Hsieh & Chen, 2009; Hsieh & Chen, 2015).

Other pertinent physical parameters for these spawning grounds include the salinity of the surrounding water, which falls within the range of 17.3 to 33.0, organic content in the range of 0.2-0.6%, dissolved oxygen in pore water at more than 4 mg L^{-1}, and a redox potential between 170 and 260 mV (Sekiguchi, 1999; Maeda et al., 2000; Ohtsubo et al., 2005).

In many studies, sedimentary properties, such as grain sizes and compositions, are most commonly used to describe the physical traits of *T. tridentatus* spawning grounds. The species spawns in a variety of sediment types with medium diameters ranging from 0.09 to 5.00 mm encompassing fine to gravelly sand (e.g., Botton et al., 1996; Wada et al., 2010; Iida et al., 2017; Mohamad et al., 2019).

Despite the widely recognised detrimental impacts of coastal development on horseshoe crab habitat contributing to population declines, there has been no detailed study to determine the causal links between coastal development and the decline in *T. tridentatus*. In the United States, it has been reported that constructing beach protection structures such as seawalls within the intertidal zone reduces *L. polyphemus* spawning habitats and the egg survival rate (Botton et al., 1988; Penn & Brockman, 1994; Jackson et al., 2015; Vasquez et al., 2015). With respect to research on *T. tridentatus* egg development, studies have been limited to animals kept in captivity (Sugita & Sekiguchi, 1981; Sugita et al., 1985; Sekiguchi, 1999). There are a few studies investigating hatching rates for *T. tridentatus* eggs in wild populations (e.g., Maeda et al., 2000; Hsieh & Chen, 2009). However, they all focused on juvenile ecology, such as the timing of larval dispersal. There have been no field-based experiments or surveys to assess the impact of coastal development on egg development in *T. tridentatus*.

While there have been studies on changes in the microhabitat of *T. tridentatus* juvenile habitats based on the interpretation of aerial photographs (Seino et al., 1998b; Seino, 2001; Seino et al., 2001a), no prior research has tracked changes in spawning habitats from a period of minimal coastal development impact and compared the findings with the results of long-term field surveys. More importantly, the previous studies mentioned above are limited to reporting on the environmental conditions of the spawning grounds where nests and eggs were found. A comprehensive analysis that integrates biological data, such as nest and egg occurrence, with relevant physical parameters has not been conducted. For instance, there is a lack of statistical analysis to determine the essential environmental factors influencing adequate nest locations for egg development. Furthermore, no scientific models have been developed to evaluate spawning ground suitability. Thus, the effect of coastal development on the *T. tridentatus* spawning habitat leading to the decline of populations and the development of scientific models to elaborate these causal relationships remains largely unexplored. Consequently, despite the alarming threat of global extinction of *T. tridentatus* populations, scientific research has not comprehensively elucidated the underlying factors responsible for this decline.

1.3 Research Objectives

This research aims to address the previously mentioned unexplored scientific aspects, provides methodologies, and draws general conclusions and insights, which can potentially be applied to the broader context of conserving *T. tridentatus* spawning grounds.

The research objectives encompassed two primary goals. Firstly, to investigate the historical impact of coastal development on the spawning grounds by tracking the spawning ground and population status over the long term in Tsuyazaki Cove, Fukuoka. Secondly, to estimate how coastal development has affected these spawning habitats and led to the population decline by performing integrated analyses that match biological data with relevant physical parameters. The objectives of each chapter are as follows.

i. To investigate the historical impact of coastal development on *T. tridentatus* spawning habitats and assess their potential implications for the species' decline (Chapter 2).

ii. To investigate the potential impact of coastal development on the spawning site selection and nesting patterns of *T. tridentatus* (Chapter 3).

iii. To analyse the relationship between egg survival rate and environmental factors using scientific models to identify suitable nesting zones for egg development and evaluate the specific impacts of coastal development on *T. tridentatus* eggs (Chapter 4).

iv. To evaluate spawning sites degraded by coastal development using the Habitat Suitability

Model (Chapter 5).

v. To propose potential conservation practices for recovering the viability of the spawning ground in Tsuyazaki Cove (Chapter 6).

1.4 Proposed Methodology and Methods

This research study adopted a comprehensive methodology that integrated historical and scientific data analysis, field surveys and experiments, together with advanced modelling techniques, to address the above specific objectives and achieve the overarching research goals.

In Chapter 2, historical changes in the spawning habitat of *T. tridentatus* in Tsuyazaki Cove were examined by analysing aerial photographs from 1948 to 2010. The changed conditions were then assessed in relation to the estimated habitat status derived from resident interviews and the spawning survey results. These data were then used to evaluate the potential impacts of these habitat changes on the species' decline. Historical changes in the spawning habitat and landfills around the bay mouth from the post-war period to the present were digitally mapped and used to calculate the impact of landfills on the spawning grounds. Since the population status of *T. tridentatus* in the study site during the post-war period remained largely unknown, an interview survey was conducted to estimate their abundance. The interviews gathered information on the year, location, and size (juvenile, sub-adult, and adult) of the *T. tridentatus* sightings and other relevant coastal environmental details. The locations of reported sightings were plotted on a map. Spawning surveys were conducted between 2005 and 2013 to determine the changes in the number of spawning pairs.

Chapter 3 established the foundation for the following chapters. This chapter focused on identifying the spatial distribution of nests and analysing environmental factors influencing nesting patterns. Specifically, the recorded nest locations were overlaid onto digital elevation maps, thus allowing for the estimation of nest elevation and slope. Moreover, sediment samples were collected from each spawning site. These variables, namely, nesting elevation, slope, and sediment types were used to assess the species' nesting environment preferences. Principal Component Analysis (PCA) was used to evaluate the selection of spawning sites and nesting patterns of *T. tridentatus*. In addition, it highlighted the underlying impact of the habitat changes on the nesting horseshoe crabs.

In Chapter 4, a field experiment was conducted by translocating *T. tridentatus* eggs to different elevations of the intertidal zone within the spawning ground in order to elucidate the relationship between the environmental variables and egg viability based on the findings from Chapter 3 (which confirmed the presence of nests/eggs in what had been considered unfavourable environments in previous research). The survival rate of *T. tridentatus* eggs translocated between high to low intertidal zones was calculated, and statistical analyses, including one-way ANOVA, were performed to identify significant differences in egg survival within the intertidal zones. The following physical parameters were collected: beach elevation, salinity, nest temperature, air exposure time, water content, and sediment samples. Generalised linear models (GLMs) were then used to assess specific physical parameters (i.e., dependent variables) affecting egg survival (i.e., predictor variable) to determine the suitability of various intertidal zones for egg development during nesting.

In Chapter 5, the suitability of spawning habitats was estimated and mapped based on scientific models and the insights derived from the findings in Chapters 2, 3, and 4. Two essential analytical methods were employed in this chapter. Firstly, generalised linear models (GLMs) were applied to evaluate the contribution rates of environmental factors to the nest/egg occurrence. Secondly, the parameters that significantly contributed to the nest/egg occurrence were further analysed using maximum entropy modelling (Maxent). In the Maxent analysis, multiple separate tests were conducted to verify the accuracy of the models by extrapolating training data to other spawning sites. The best model was then applied to generate the spawning suitability map of the area.

1.5 Description of Study Site

This research was conducted at Tsuyazaki Cove (33 ° 47'N, 130 ° 27'E) in Fukuoka, Japan (Figure 1.2). The cove is an inland lagoon with geographical isolation from the open sea, encompassing an approximate circumference of 4 km. The tidal range adjacent to Tsuyazaki measures approximately 1.7 m during spring tides (Japan Coast Guard, 2022). The cove, formerly an expansive inner bay and salt marsh, underwent significant transformations due to landfilling for salt farming during the 17th and 18th centuries (Hirowatari & Shimoyama, 1999). Consequently, most of its original expanse was landfilled, leaving remnants of salt farms and agricultural areas (most of which have been converted into mega-solar facilities since 2013) that now constitute the cove's eastern hinterland, and its western periphery features a compact mountainous coastline (Figure 1.2).

Tsuyazaki Cove lies between the two urban centres of Fukuoka and Kitakyushu cities. Despite considerable developmental activities in its vicinity, it continues to serve as a crucial inner bay within the Fukuoka metropolitan region. Notably, the area provides habitat to endangered species spanning diverse taxonomic categories, including the black-faced spoonbill (*Platalea minor*), the chikuzen goby (*Gymnogobius uchidai*), and the tri-spine horseshoe crab (*Tachypleus tridentatus*). Furthermore, the cove and its surrounding salt farms and agricultural areas have been designated as an "Important Wetland for Biodiversity in Japan" by the Ministry of the Environment. It plays a crucial role as a habitat and migratory route for shorebirds. The wetland is also the habitat of the endangered freshwater fish, Rosy bitterling (*Rhodeus ocellatus kurumeus*) (Itaya et al., 2018).

A number of studies on *T. tridentatus* have been carried out within Tsuyazaki Cove. These encompass the juvenile population and the suitability of juvenile habitat, examination of the adult habitat and movement patterns as well as the spawning population (Wada et al., 2008; Wada et al., 2010; Koyama et al., 2020). A habitat for juvenile *T. tridentatus* has been confirmed on the sandy mudflat on the cove's northern periphery (Wada et al., 2008). The spawning behaviour of *T. tridentatus* has been documented within restricted sandy beaches along Tsuyazaki Cove, with an estimated 70% of the spawning instances concentrated in proximity to sites adjacent to Tsuyazaki Bridge (Figure 1.2) (Wada et al., 2010). The coastal development of Tsuyazaki Cove gradually progressed with the rapid post-war economic growth and continued into the 2000s. Most recently, the construction of a yacht harbour was completed in 2005 and subsequently, a breakwater at the cove entrance was established in 2006. The latter was built to protect the yacht harbour facilities but also blocked the only opening of the cove (Figure 1.2). Implementing these coastal development infrastructures has coincided with a pronounced reduction in *T. tridentatus* spawning numbers within the area. Consequently, a pressing imperative for knowledge exists in order to unravel the causal linkage between coastal development and the decline in the *T. tridentatus* population in Tsuyazaki.

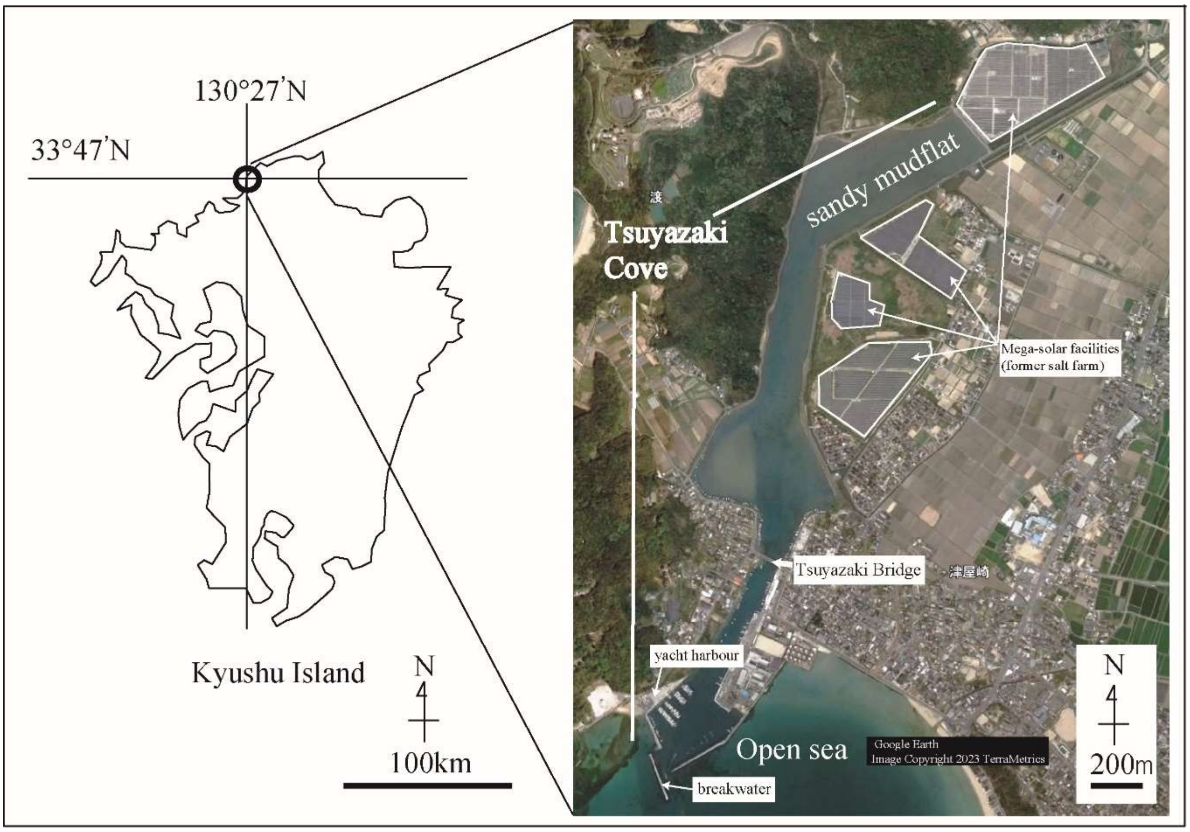

Figure 1.2 Study site: Tsuyazaki Cove, Fukuoka, Japan. Dispersed along Tsuyazaki Cove, small sandy beaches are the spawning grounds for *T. tridentatus*. In particular, the sandy beaches adjacent to Tsuyazaki Bridge are of paramount importance, hosting the local population's predominant share of spawning activities. The sandy mudflat north of the cove is known as a *T. tridentatus* juvenile habitat.

1.6 Dissertation Structure

This dissertation comprises six chapters (Figure 1.3). Chapters 2, 3, 4, 5 and 6 are based on published studies in international journals.

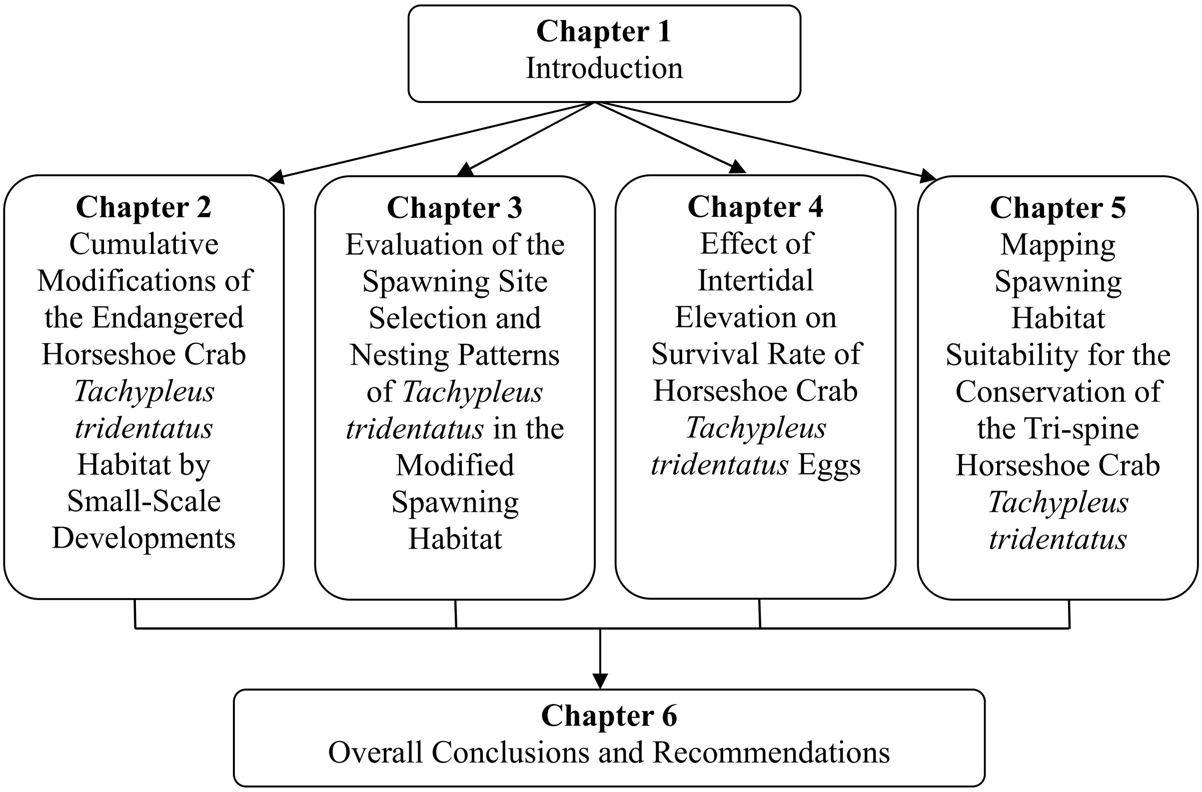

Figure 1.3 Dissertation structure

Chapter 1: Introduction. This opening chapter presents the research background, what is known in previous studies and their shortcomings while emphasising the significance of the current research. The research objectives are clearly outlined, and a detailed description of the study site is provided. Lastly, the dissertation structure is presented.

Chapter 2: Cumulative Modifications of the Endangered Horseshoe Crab *Tachypleus tridentatus* Habitat by Small-Scale Developments. This chapter compiles the research findings from the published papers in Itaya et al., 2019b and 2022b. The chapter reports the research undertaken into historical changes to the spawning habitat of *T. tridentatus* in Tsuyazaki Cove from 1948 to 2010 by examining aerial photographs. Subsequently, these changes were considered with respect to personal interviews with long-standing residents and the monitoring of spawning pairs to gain insights into the factors behind the decline of *T. tridentatus*. Of major significance is the finding that coastal development modifications to the cove entrance led to the disruption of sediment transport routes and, subsequently, the degradation of spawning habitats in the study area.

Chapter 3: Evaluation of the Spawning Site Selection and Nesting Patterns of *Tachypleus tridentatus* in the Modified Spawning Habitat. This chapter is derived from the research findings from the published paper in Itaya et al., 2019a. Chapter 3 documents the research into the spawning site selection and nesting patterns of *T. tridentatus* and reveals how modification of natural beach shapes led to significant changes in nesting. The aim was to confirm personal field observations that *T. tridentatus* seemed to lay eggs in lower intertidal zones, which were previously considered unsuitable for spawning. This shift in egg deposition was found to occur after the original beach shape had been altered by coastal development. The study of this chapter revealed the significance of the original shapes of beaches in providing favourable spawning habitats. Chapters 4 and 5 further consider the implications of this chapter.

Chapter 4: Effect of Intertidal Elevation on Survival Rate of Horseshoe Crab *Tachypleus tridentatus* Eggs. This chapter is constructed from the research findings published in Itaya et al., 2022a. *T. tridentatus* is typically known to nest in the intertidal zone, specifically between the mean higher high water (MHHW) and the mean high water neap (MHWN). However, there have been instances where nesting occurs at lower elevations than the MHWN, particularly within spawning sites impacted by coastal development. Chapter 3 confirmed similar nesting behaviour. Although previous studies on Atlantic horseshoe crabs reported low egg survival rates in unfavourable environments, no field-based research had investigated the favourability of different elevations across the high to low tide zones on *T. tridentatus* eggs. Therefore, this chapter focuses on a study of egg survival at different elevations as a way of testing the hypothesis that eggs laid at unfavourable elevations exhibit lower survival rates than those laid at normal (favourable) elevations. The study of this chapter investigated and analysed the factors influencing egg development and survival in different intertidal zones, and the possible impact of coastal development on egg survival was discussed.

Chapter 5: Mapping Spawning Habitat Suitability for the Conservation of the Tri-spine Horseshoe Crab *Tachypleus tridentatus*. This chapter concerns the research findings from the published paper in Itaya et al., 2023. It considers the research that examined the suitability of the remaining spawning sites using scientific models. The study aimed to further assess the potential impact of coastal development on *T. tridentatus* nests and eggs by identifying "suitable" and "unsuitable" spawning zones. The study of this chapter showed the critical role of beach elevation relative to tidal range in predicting nest/egg occurrence. It demonstrated that around MHWN (Mean High Water Neap: the mid intertidal zone) was the most suitable for nesting, consistent with the high egg survivability found in Chapter 4. The study also revealed that the suitable spawning habitat area was estimated to constitute only 24% of the sandy beach within the study site, which is attributed to habitat fragmentation caused by cumulative coastal development since the post-war period.

Chapter 6: Overall Conclusions and Recommendations. This final chapter consolidates the research outcomes presented in the published papers by Itaya et al. in 2019b, 2022a, 2022b, and 2023. It also synthesises the conclusions reported in each of the research studies. A range of conservation measures are proposed based on the findings, giving emphasis to the conservation and restoration of suitable spawning habitats to facilitate the population recovery of *T.*

tridentatus in Tsuyazaki Cove. Conservation strategies proposed include protecting remaining spawning grounds, beach nourishment, restoring sand supply and implementing legal regulations. Additionally, further research beyond the scope of this dissertation is recommended, such as a detailed analysis of tidal current changes resulting from artificial shoreline modification and exploring effective methods for conserving and recovering the remaining spawning sites.

Throughout this research study, numerous field-based investigations, careful data analysis, and specific modelling techniques were undertaken to systematically investigate the decline of *T. tridentatus* and explore possible conservation strategies in Tsuyazaki Cove. By synthesising the findings from each chapter, this research aimed to advance ecological understanding and conservation practices regarding this globally endangered species.

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Chapter 2

Cumulative Modifications of the Endangered Horseshoe Crab *Tachypleus tridentatus* Habitat by Small-Scale Developments

Abstract Possible factors causing the decline of an endangered horseshoe crab species, *Tachypleus tridentatus*, were revealed by the interpretation of aerial photographs, interviews with the local people and field surveys at Tsuyazaki Cove in Fukuoka, Japan. By the interview of the locals, it was confirmed that *T. tridentatus* seemed to be abundant in the area from the 1940s to 1980s; however, the species gradually decreased and were scarcely seen by the locals in the early 2000s. The field survey from 2005 to 2013 confirmed that the number of spawning horseshoe crabs dramatically reduced over the survey period. The results of this chapter suggest that the decline of *T. tridentatus* can be explained in relation to the reclamation of the bay mouth, causing poor sand supply to potential spawning sites, which in turn resulted in the habitat degradation of local populations of *T. tridentatus*. The historical ecology approaches applied in this chapter could be useful as a primary analysis to search for clues of the causal relationship between the population declines of rare species in hand and coastal developments.

Keywords: coastal development, endangered species, environmental conservation, habitat degradation, historical ecology

2.1 Introduction

Tachypleus tridentatus, commonly known as the tri-spine horseshoe crab, is one of the four extant species of horseshoe crabs found along coastal zones in East and Southeast Asia (Sekiguchi, 1989; Sekiguchi, 1999). The spawning of *T. tridentatus* in Japan mainly occurs from late June to August (Sekiguchi, 1999; Wada et al., 2010).

In Japan, *T. tridentatus* is listed as Critically Endangered (Ito, 2014). After researching the four species of horseshoe crabs throughout the world, Sekiguchi (1989: p.17) concluded that "there are no other horseshoe crabs' habitats in the world which have been devastatingly destroyed as those in Japan as a result of development." Habitat loss, particularly caused by the development of coastal zones for economic growth to increase industrial and agricultural production, has resulted in decreasing their populations. Although other factors (e.g., water pollution and changes in substratum condition due to dam constructions that restricted river discharges) are responsible for the species decline, habitat loss is the major problem in many cases in Japan and other Asian countries (Sekiguchi, 1989; Sekiguchi, 1999; Akbar John et al., 2018). In 2019, *T. tridentatus* was assessed as endangered by the International Union for Conservation of Nature (Laurie et al., 2019). Thus, the species is facing the risk of extinction not only in Japan but also globally, so urgent conservation practices are crucial.

Coastal areas where horseshoe crabs inhabit are also sites for human activities, such as fisheries, and are therefore vulnerable to various anthropogenic impacts. In recent years, the importance of conserving coastal environments has increased, and the conventional prioritization of civil engineering projects over conservation has been called into question, emphasising the need to develop strategies that promote both conservation of endangered species such as horseshoe crabs and sustainable development (Seino et al., 2000). In particular, conservation and restoration of sandy beach environments that serve as spawning grounds are crucial tasks, as the declining population of horseshoe crabs has become a significant issue (Wada et al., 2010).

In Tsuyazaki Cove, Fukuoka, a study has been conducted on the conservation of spawning sites, which reports on the selection of spawning sites and the annual changes in the number of spawning pairs (Wada et al., 2010). According to Wada et al. (2010), the number of spawning pairs in Tsuyazaki decreased every year during the survey period from 2005 to 2008 and understanding the causal relationship between the decrease in the number of individuals and coastal development has become an issue. The decline of *T. tridentatus* in various parts of Japan is largely due to the disappearance of their habitats caused by reclamation projects during the economic growth periods (Nishii, 1975; Souji, 1992; Sekiguchi, 1991; Seino, 2001). A similar phenomenon may have occurred in Tsuyazaki, and it is considered necessary to investigate the impact of coastal development on the population at that time. However, no previous studies have tracked the deterioration of the habitat, especially the changes in the sandy beach as a spawning ground for *T. tridentatus* in the area.

Thus, in this chapter, the *T. tridentatus* population in Tsuyazaki Cove was examined through interviews to determine their habitat status from the post-war period to the present day, as well as field surveys to estimate the number of spawning pairs. The changes in the sandy beach as a spawning ground for *T. tridentatus* were also analysed. Finally, the reduction in the *T. tridentatus* population in the area and the reasons for this reduction in relation to coastal development were discussed.

2.2 Methods and Materials

2.2.1 Study Site

The study of this chapter was conducted at Tsuyazaki Cove, Fukuoka (Figure 2.1). Tsuyazaki Cove is a lagoon-type inner bay formed on the residual sand dunes of the Holocene epoch. There are no major river inflows into this cove. Tsuyazaki Cove was once a vast bay with salt marsh, but salt field reclamation began during the seventeenth and eighteenth centuries, filling most of the area (Figure 2.2) (Hirowatari & Shimoyama, 1999). Currently, the remaining salt field remnants (most of which have been converted into a mega-solar facility since 2013) and farmland form the hinterland of the cove.

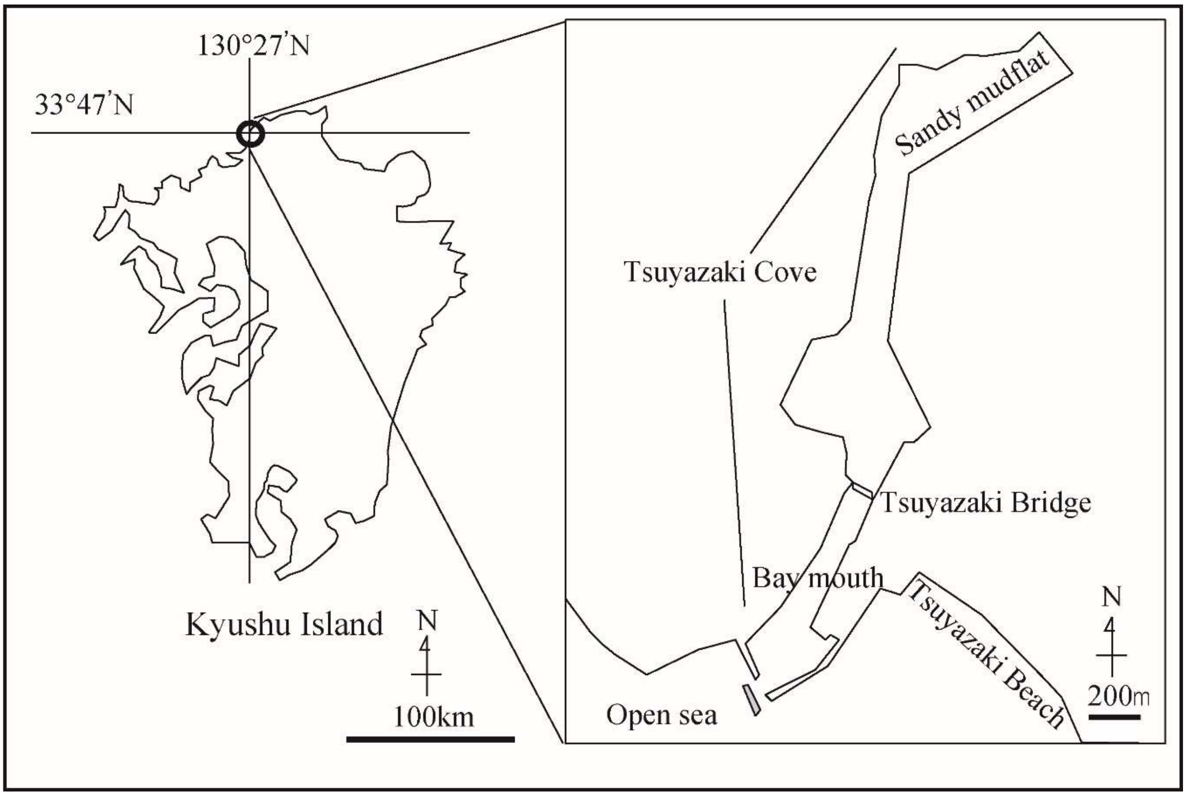

Figure 2.1 Location of the study site in Tsuyazaki, Fukuoka, Japan.

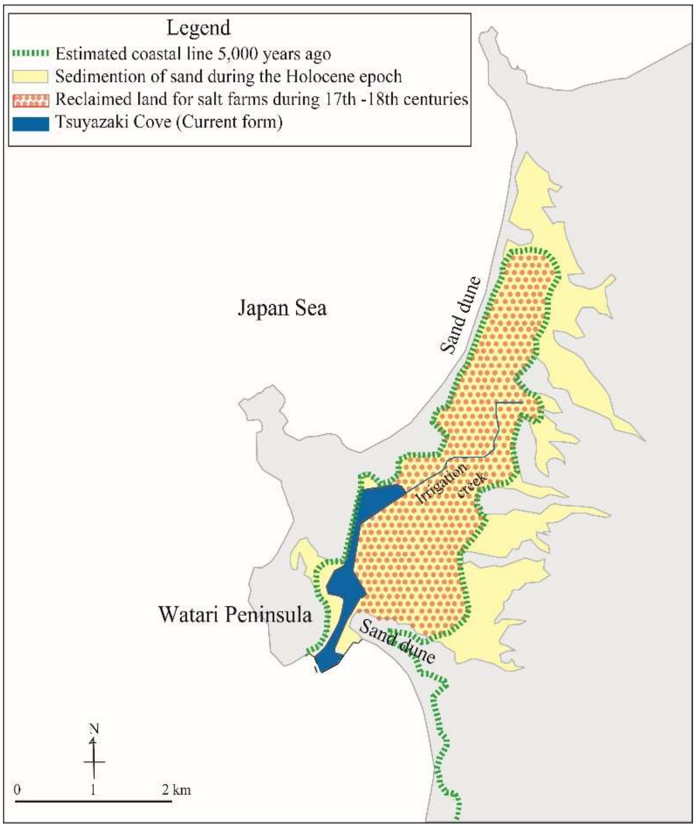

Figure 2.2 Geographical feature of Tsuyazaki Cove vicinity (modified from Hirowatari & Shimoyama, 1999).

2.2.2 Changes in the Bay Mouth over time

The study of this chapter focused on the Tsuyazaki Bay mouth, where approximately 70% of *T. tridentatus* spawning occurs (Wada et al., 2010). To investigate the factors leading to a decline in *T. tridentatus* spawning pairs, alterations to the bay mouth and changes in the sandy beach as a spawning substrate were tracked using aerial photographs. Previous studies have indicated that suitable areas for *T. tridentatus* spawning are sandy beaches between the highest tide line of spring and the neap highest tide line (Seino et al., 1998; Sekiguchi, 1999). In addition, according to Wada et al. (2010), the sediment at the Tsuyazaki spawning area consists of sand with particle sizes ranging from fine sand (0.125 to 0.25 mm in diameter) to gravels (4.0 mm or larger in diameter) with a silt-clay content of 0.0 to 6.1%. Based on these reports, the interpretation of aerial photographs in this chapter defined spawning grounds as sandy areas located above the midpoint between the low and high tide lines in the intertidal zone. Figure 2.3 shows an example of the spawning beach definition used in this chapter. Site 2 is shown here as an example because it is the only site where the highest tide line remains in Tsuyazaki. Aerial photographs taken by the Geospatial Information Authority of Japan in 1948, 1961, 1966, 1979, 1981, 1990, 2003, and 2010 were used for photo interpretation. Aerial photographs were selected at equal time intervals whenever possible. However, due to certain photographic conditions, such as sunshine reflections, some sandy beaches could not be clearly identified. In such cases, only photographs with easily identifiable sandy beaches were used, excluding those that presented difficulties. The deciphered bay mouth modifications and changes in the sandy beach were digitised, and their areas were calculated using QGIS version 2.18. The representative years 1948, 1966, 1979, 1990, 2003 and 2010 were selected, and schematic diagrams were made for these photos using Adobe Illustrator version 10.

For Site 2, which was reported by Wada et al. (2010) to have the highest spawning activity in Tsuyazaki, a comparison was made between photographs taken during the 2007 field survey and those taken in 2018 to observe changes in the shape of the beach. The height of the sand deposits was estimated based on the photographs and field observations, specifically by assessing the relative height of the sand deposit in relation to the revetment. Additionally, the reduction in beach height resulting from runoff was measured in October 2018.

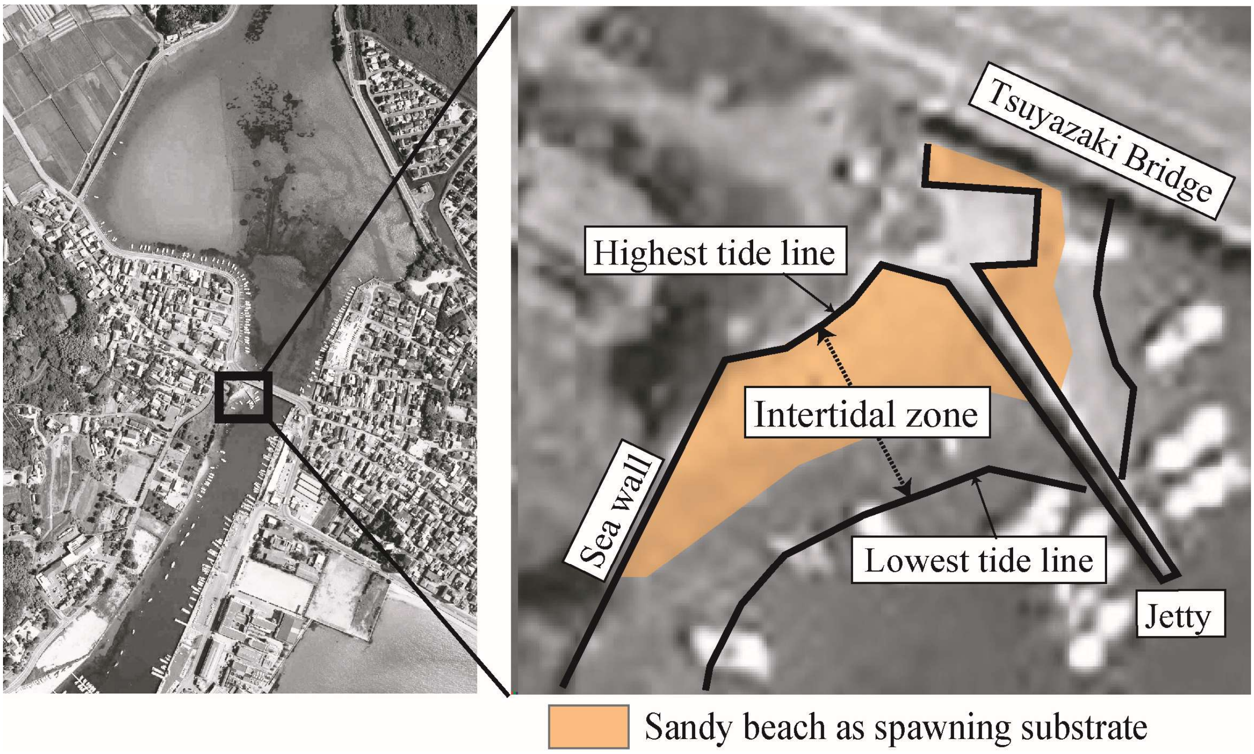

Figure 2.3 Definition of sandy beach as spawning substrate in this chapter. Site 2 is shown in the figure as the most typical spawning ground in this chapter. The aerial photographs were obtained from the Geospatial Information Authority of Japan. https://mapps.gsi.go.jp/maplibSearch.do#1

2.2.3 Interview Survey

The distribution and abundance of *T. tridentatus* in Tsuyazaki from the post-World War II period to the present were obtained through interviews conducted with local residents. The interviews were conducted between January and February 2018. The study of this chapter targeted ten individuals aged 45 years or older at the time of the interviews. The participants included local fishermen and members of the Fukutsu Historical Society. Individuals younger than 45 years old were excluded from the interview since their sighting information overlapped with the author's field survey period and was considered too recent. These individuals were interviewed to gather testimony regarding the year, location, and size (juvenile, sub-adult, and adult) of the *T. tridentatus* sightings, as well as other information related to the coastal environment at the time. To determine the size of *T. tridentatus*, the participants were shown photographs depicting the size of each growth stage and asked to estimate the size of the *T. tridentatus* they had observed. The reported sighting locations were then plotted on a map.

2.2.4 Spawning Survey

Spawning surveys were conducted at the survey sites shown in Figure 2.4 from late June to early August between 2005 and 2013. The surveys were carried out continuously for 3 or 4 days during consecutive high tide periods, totalling 10 or 11 days each year, with exceptions for cases of typhoons or thunderstorms to ensure the safety of the surveyors. Overall, the surveys were conducted for 89 days, averaging 8.9 days per year. Each survey was conducted for three hours, from two hours before the highest tide to one hour after. Surveys were carried out twice a day, once in the morning and once in the evening, resulting in six hours of surveying per day. The survey was conducted by a team of surveyors, including the author, students from Fukuoka ECO Communication College (now Fukuoka College of ECO and Animals) and resident volunteers. The average number of surveyors was 8.9, ranging from 1 to 18 individuals. Prior to the survey, the author provided introductory lectures to the surveyors to standardise the survey techniques and explain the significance of the study of this chapter.

During the surveys, the spawning sites along the cove were patrolled by surveyors on foot or by bicycle at high tides to find *T. tridentatus* spawning pairs. National BF-153B underwater lights were used to search for horseshoe crabs for nocturnal surveys. When spawning horseshoe crabs were discovered, they were gently restrained in the water after completing spawning and marked for individual identification. To mark the horseshoe crabs, a white crayon manufactured by Sakura Color Products Corp. was used along with tags. Each horseshoe crab was marked with a unique number on the sides of its carapaces using the crayon. Subsequently, a tag containing a paired identification number was attached to the telson of the male. In addition, the attachment of barnacles on the carapaces and the presence of exoskeleton injuries were also documented on the survey sheet as additional characteristics for individual identification. The number of spawning pairs in each year was determined by excluding duplicate counts based on individual identification.

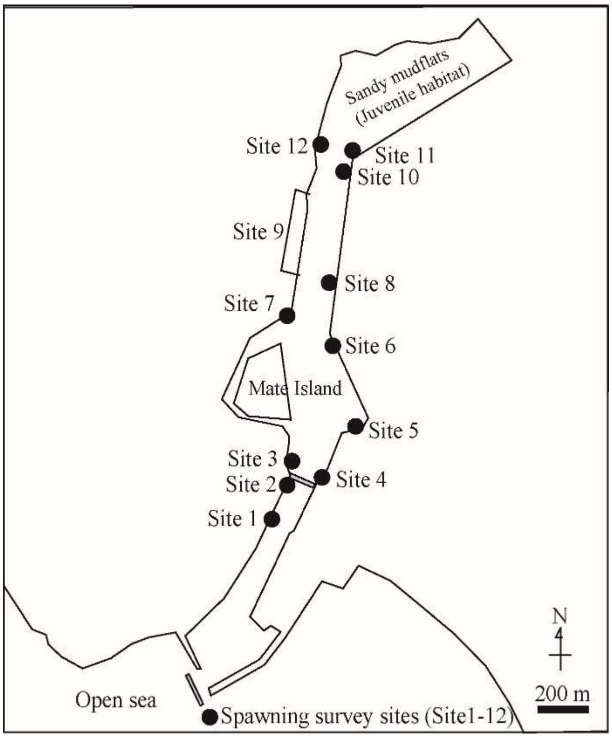

Figure 2.4 *T. tridentatus* spawning survey sites in Tsuyazaki Cove.

2.2.5 Data Analysis

Statistical analyses were performed using Microsoft Office Excel 2007 and R version 3.2.1. For changes in the cove mouth development over time, the relationship between the landfilled and the sandy beach areas was compared by determining Pearson's correlation coefficient. Pearson's chi-square test was performed for the comparison of the habitat preference between the sites. The Mann-Whitney U-test was employed to determine significant differences in the number of *T.*

tridentatus spawning pairs before and after 2009, given the observed substantial decline in pairs following 2009.

2.3 Results

2.3.1 Bay Mouth Modification and Reduction of Sandy Beaches from 1948 to 2010

Figures 2.5, 2.6, 2.7 and Table 2.1 show the bay mouth modification in Tsuyazaki Cove from 1948 to 2010 and the associated changes in the sandy beaches over time (The mesh numbers in Figure 2.5 correspond to Table 2.1).

In 1948, there was little development at the mouth of the bay. The bay mouth was widely opened to the sea, with only a 300 m long jetty on the left bank. The sandy beach was continuous from the mouth of the bay to Mate Island, mainly on the right bank (Figure 2.6a). This beach continuity was maintained to some extent until 1966 (Figure 2.6b). Mate Island was excavated in 1969, resulting in the straightening of the channel (Figure 2.5). Subsequently, part of the cove road was constructed during the replacement of the Tsuyazaki Bridge in 1978, which resulted in the near disappearance of the sandy beach on the southwest side of Mate Island in 1979. Furthermore, the sandy beach downstream of the Tsuyazaki Bridge on the right bank also decreased during the same period (Figure 2.6c). Between the 1980s and 1990s, the bay mouth was narrowed by land-filling, including the construction of the yacht harbour on the right bank, almost leading to the original shape of the present bay mouth. Sand accumulation has begun on the western side of the yacht harbour since this period (Figure 2.6d). This sand accumulation increased between 2003 and 2010 (Figures 2.5e and 2.6f), and in 2006 a breakwater was completed to block the bay mouth, leaving only a space of about 60 m at the contact point between the bay mouth and the open sea. Thus, the spawning grounds of *T. tridentatus* experienced a significant decline in the 1970s, and subsequent coastal development has further diminished them. The remaining spawning grounds of *T. tridentatus* now comprise only a few scattered sandy beaches (Figure 2.6f).

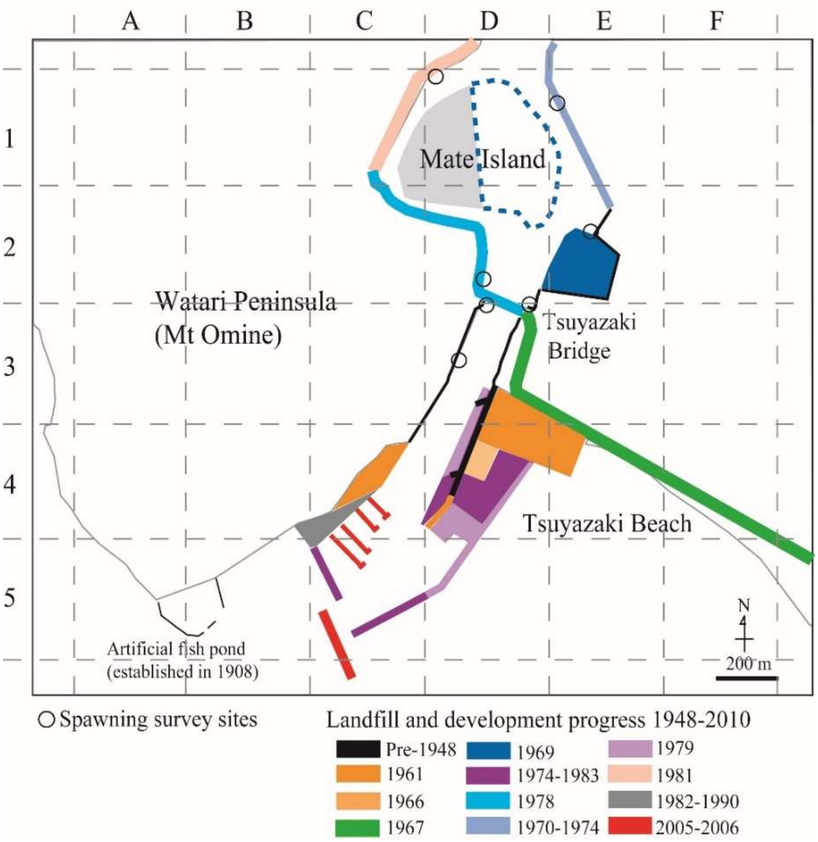

Figure 2.5 Changes in the modification of the bay mouth at Tsuyazaki Cove from 1948 to 2010.

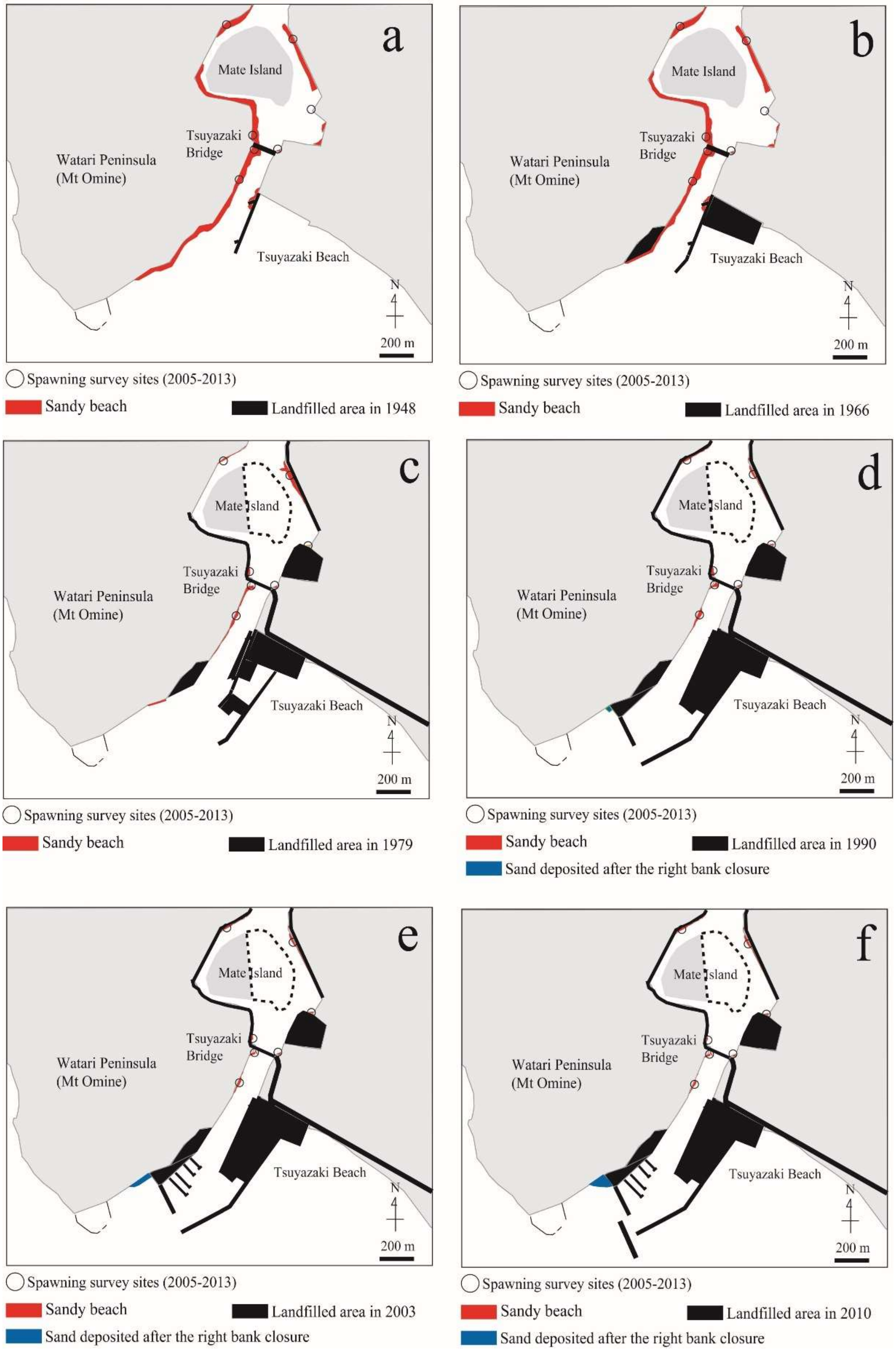

Figure 2.6 Changes in spawning beaches associated with bay mouth modification in Tsuyazaki Cove during 1948-2010.

Table 2.1 The spawning beach conditions and the modification of the bay mouth in Tsuyazaki Cove from 1948 to 2010.

Year	Construction year	Mesh number in Figure 2.5	Modification of the bay mouth	Sandy beach area (m^2)	Sandy beach conditions
1948		D3, D4	There was a jetty of about 300 m on the left bank.	26,303	There were no artificial structures on the Cove's right bank, and the sandy beach's continuity was maintained.
1961		D4	The jetty on the left bank side was extended seaward by about 100 m.	25,158	Coastal development reduced the sandy beach area by 4.4% compared to 1948, but the continuity of the sandy beach on the right bank was maintained.
		D3, D4, E4	The left bank of the Tsuyazaki beach was partially reclaimed.		
		C4	Partial reclamation of the bay mouth on the right bank.		
1966		D4	Slight extension of landfill on left bank side.	25,038	Coastal development on the left bank had little impact (beach area decreased by 0.5% compared to 1961), and the continuity of the right bank beaches was maintained.
	1967	F4, E4, E3, D3	Tsuyazaki coastal road was completed.		
	1969	E2	The back left bank side was reclaimed by housing development.		
		D1, D2	Mate Island was excavated due to construction work.		
	1974	D4	Left bank landfill works started and were completed in 1983.		
	1978	D2, C2	Tsuyazaki Bridge replacement (partial construction of attached road).		
1979		D4, D5	Landfill works on the left bank proceeding.	13,547	The construction of the road around the Cove significantly reduced the continuity of the sandy beach on the right bank. The sandy beach area significantly decreased to 45.9% compared with that in 1966.
1981		C1	The Cove perimeter road was completed.	9,168	The beach area decreased by 32.3% compared to 1979.
	1982	B4, C4	Land reclamation of the right bank for a yacht harbour started and was completed in 1990.		
	1983	D4	Reclamation works on the left bank were completed.		
		C5	The jetty on the left bank was extended about 150 m offshore.		
		C5	A jetty of about 130 m at the entrance to the bay was completed.		
1990	1990	B4, C4	Land reclamation for the yacht harbour site was completed.	6,858	The beach area decreased by 25.2% compared to 1981 resulting in the fragmentation of sandy beaches on the right bank.
		B4, B5, C4, C5	The tidal channel on the right bank was blocked.		
		C5	The contact zone between the Cove and the open sea was reduced to about 100 m.		
2003		C4, C5	The yacht mooring area was under construction.	6,391	Reclamation was almost completed in 1990, and there was no rapid increase in the reclaimed area in the 2000s. The sandy beach area decreased by 6.8% compared to 1990.
	2005	C4, C5	The yacht harbour construction work was completed.		
	2006	C5	A breakwater was constructed at the bay entrance, reducing the water contact area between the Cove and the open sea to about 60 m.		
2010				5,376	No major reclamation other than a breakwater, but the beach area was reduced by 15.9% compared to 2003. The continuity of the sandy beach has disappeared.

As mentioned above, the development of the bay mouth area progressed during rapid post-war economic growth. The coastal landfill area expanded from 1,954 m^2 in 1948 to 88,394 m^2 in the 1970s. After the 1970s, development continued, resulting in a total landfilled area of 118,681 m^2 in 2010, the largest in the post-war period. In contrast, the sandy beach area decreased over time due to the expansion of the coastal landfill, ranging from a maximum of 26,303 m^2 in 1948 to a minimum of 5,376 m^2 in 2010 (Figure 2.7). A negative correlation was found between the area of the spawning beach and the landfill area. The *T. tridentatus* spawning ground significantly decreased as the coastal landfill progressed (Pearson's correlation r =-0.96, p<0.05).

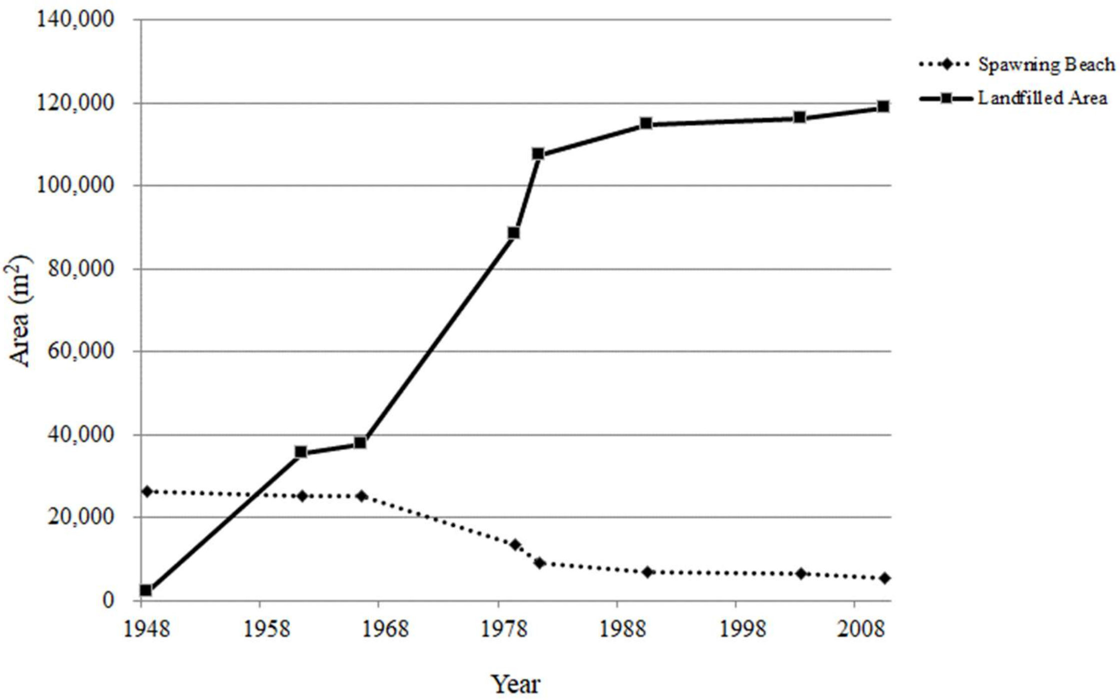

Figure 2.7 Comparison between the area of the potential spawning beach of *T. tridentatus* and area of the landfills from 1948 to 2010 in Tsuyazaki.

Figure 2.8 shows the changes in the sandy beach at Site 2 between 2007 and 2018. In 2007, the west side of the jetty exhibited a substantial coverage of sand, with more than half of the stone wall being concealed. A clear continuity was observed between the vegetation zone and the sandy beach. However, by 2018, significant sand erosion had occurred, resulting in the exposure of the stone wall and the disappearance of approximately half of the vegetation zone. The width of the sand erosion ranged from 53 cm (maximum) to 20 cm (minimum) (Figure 2.8b). Furthermore, most of the smaller sand particles were washed away, leaving only pebble-sized particles on the northern side of the jetty (Figure 2.8c).

Figure 2.8 Comparison of beach morphology and condition at Site 2 between 2007 and 2018. (a) Birds-eye view, showing the two spawning areas, b and c. (b) Comparison of the sand accumulation at b area in 2007 (above) and in 2018 (below). (c) Comparison of the sand accumulation at c area in 2007 (left) and in 2018 (right).

2.3.2 *T. tridentatus* Population Status from 1940s to 2010 based on Interview Surveys

In the interview survey, testimonies and location information on *T. tridentatus* from ten residents born between 1939 and 1972 were collected (Table 2.2 and Figure 2.9). Based on the testimonies, it was indicated that the local community has been aware of *T. tridentatus* since the 1940s. During the post-war period until the 1980s, there were regular sightings of *T. tridentatus* in Tsuyazaki Cove. Fishermen recounted their experiences of capturing numerous *T. tridentatus* as unintentional catches in fishing nets along the coastal areas of the Watari Peninsula, and residents recalled that *T. tridentatus* caught as unintentional catches were discarded in piles around the Tsuyazaki Fishing Port until the 1980s. During that period, residents considered *T. tridentatus* a common and abundant species, while fishermen viewed them as a nuisance due to their tendency to damage fishing nets. The gathered testimonies provided compelling evidence of the substantial presence of *T. tridentatus* during that time, with statements such as "there were so many of them," "I saw them frequently," and "they were everywhere." Based on these testimonies, it was inferred that a significant population of *T. tridentatus* inhabited the Tsuyazaki coast from the post-war period to the 1980s, although the exact number of individuals was not determined. However, fishermen reported a gradual decline in their population, and by around 2008, they were rarely caught in fishing nets.

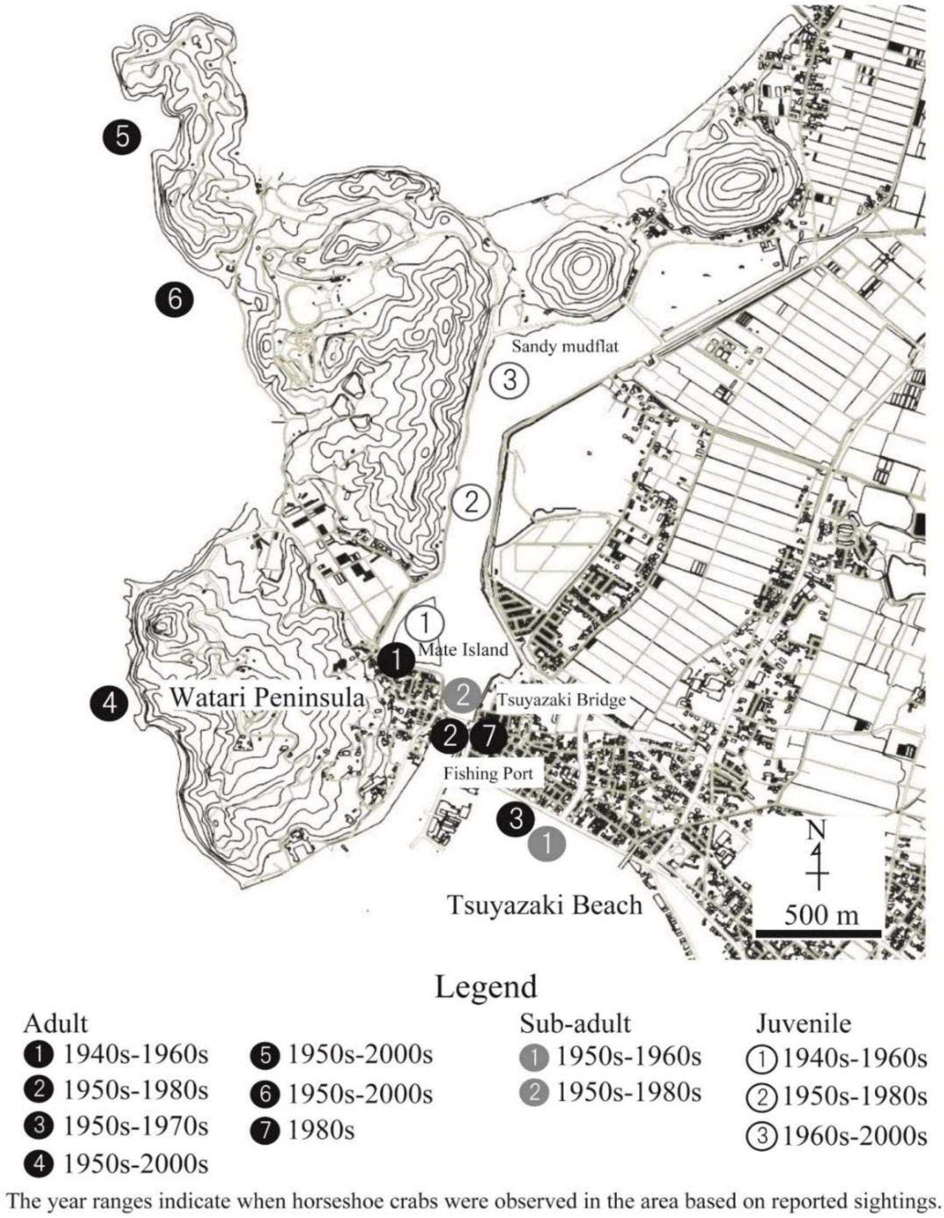

Figure 2.9 *T. tridentatus* distribution around Tsuyazaki Cove based on the interview survey. Refer to Table 2.2 for details on reported sightings. The elevation tiles were obtained from the Geospatial Information Authority of Japan. https://fgd.gsi.go.jp/download/mapGis.php

Table 2.2 *T. tridentatus* inhabitance status around Tsuyazaki Cove from the 1940s to 2000s based on the interview survey.

Period	Sighting location	Location no. in Figure 2.9	Estimated growth stage	Testimonies on *T. tridentatus* and the coastal environment
1940s	Tsuyazaki Cove (around Wateri)	①❶	Juvenile & Adult	Around 1947-48, a large amount of rubbish was dumped in the Cove, yet horseshoe crabs still inhabited the sandy beaches. At that time, more horseshoe crabs were in the Cove compared to the present. Horseshoe crabs were observed laying eggs at the water's edge during high tide. Juvenile horseshoe crabs were also found on Mate Island. Before the construction of the new road, the embankments were traditional stone walls instead of concrete, as seen today.
1950s	Tsuyazaki Beach, Tsuyazaki tidal flat & around Tsuyazaki Bridge	②➊➋ ❷❸❹	Juvenile to Adult	My father, a fisherman, used to catch many horseshoe crabs as bycatch during that period. The Tsuyazaki tidal flat and beach were home to many horseshoe crabs of various sizes. Mate Island was formerly used for razor clam farming (known as "Mate-gai" in Japanese), which is how the island earned its name. The local community also cultivated Japanese Nori (seaweed) in the Cove.
	Tsuyazaki Beach	➊❸ ❹❺❻	Subadult & Adult	During that period, subadult and adult horseshoe crabs were abundant on Tsuyazaki Beach, and people used to play with them. Horseshoe crabs were also commonly caught while digging for razor clams; some people even brought them home. Although it's unclear how many horseshoe crabs existed during that time, they could be found everywhere. However, when the author organized a horseshoe crab observation meeting in 2007, it was revealed that the number of horseshoe crabs had significantly decreased, surprising local people who had thought they were still common and plentiful. In the past, local children would collect shellfish from the sea for dinner, and Tsuyazaki Beach was abundant with shellfish. The water quality of Tsuyazaki Beach has improved since then, as in the past, sewage from the beach and tidal flats drained into the sea, causing mud accumulation in some parts of the beach.
	Watari Peninsula		Adult	Large horseshoe crabs were regularly caught in fishing nets around the Watari Peninsula.
1960s	Tsuyazaki Beach & Tsuyazaki tidal flat	③❸	Juvenile & Adult	Horseshoe crabs were frequently observed on Tsuyazaki Beach and Cove. When I was a child, I used to catch large horseshoe crabs in the Cove and take them to a mountain in Wakamiya (now Miyawaka City, Fukuoka) to swim in the river and play with them. Horseshoe crabs were my playmates during my childhood. Small horseshoe crabs were also observed in the middle of the tidal flat.
	Tsuyazaki Beach, Tsuyazaki tidal flat & around Tsuyazaki Bridge	③➊ ❷❸	Juvenile to Adult	During the late 1950s and 1960s, it was common to see horseshoe crabs on Tsuyazaki Beach and Cove, including individuals as large as 30 cm wide and smaller ones could find too. They were everywhere, and mating horseshoe crabs could be observed around the Tsuyazaki Bridge and Tsuyazaki Beach. Adult horseshoe crabs were also frequently present around the Tsuyazaki Bridge. The bank around the Cove was a natural bank with bamboo bushes before it was made of concrete, and the water was deeper around the Tsuyazaki Bridge, where people would jump in and play. Between 1955 to 1965, the bay entrance was wider, allowing many fish to enter. However, the bay entrance is now narrower, and fish cannot enter as easily.
	Tsuyazaki Cove (around Wateri)	❶	Adult	I have been living in Watari since the late 1960s. Before the construction of Cove Road, there was a sandy beach with reeds and other grasses. We used to fish and swim in that area, and there were also horseshoe crabs. At that time, there was no sealed road around the Cove, and the main road was through the alley on the south side. Later, when the Tsuyazaki Bridge was rebuilt in 1978, the road on the Watari side (southwest side of Mate Island) was constructed, and the sandy beach disappeared due to reclamation work.
1970s	Tsuyazaki Beach	❸	Adult	When I was in primary school, I found mating horseshoe crabs on Tsuyazaki beach and played with them, separating the female from the male.
1980s	Tsuyazaki fishing port	❼	Adult	When I was in primary school, I remember seeing horseshoe crabs that were bycaught in nets being piled up and thrown away at the Tsuyazaki fishing port every year. At the time, I wondered why horseshoe crabs, which seemed so abundant, were designated as a protected species and considered valuable.
	Tsuyazaki fishing port	➋❷	Subadult & Adult	When I was younger, I remember seeing many horseshoe crabs in the waters near the Tsuyazaki fishing port. Horseshoe crabs floated up in the waves as the boats came and went into the Tsuyazaki fishing port, and they were being scooped up in nets. The horseshoe crabs were around 20 cm wide, with some as big as 30 cm wide in the Cove. As my parents were fishmongers and kept horseshoe crabs in their fish ponds, I was familiar with them and didn't consider them rare. I wondered why such a large number of horseshoe crabs were declared a protected species.
	Tsuyazaki tidal flat	②	Juvenile	While digging for shells, we frequently encountered small horseshoe crabs in the past. The area also used to have an abundance of prawns and mussels, but that is no longer the case.
	Tsuyazaki fishing port	❼	Adult	During the 1980s, discarded horseshoe crabs were commonly found in piles at the Tsuyazaki fishing port, usually about 20-30 per heap. I used to think they were a nuisance for the fishermen as they got in the way of their fishing activities. Back then, I only considered horseshoe crabs as troublesome creatures. In the past, the Tsuyazaki beach was abundant with prawns, mussels, and shells, but it has not been the same for the last decade. The beach also seems narrower than before, and I heard from the fishermen that the tidal current changed since the breakwater construction at the bay entrance. My father was the chairman of the tourist association. He shared with me that the community put a lot of effort into promoting tourism during the 1970s, including building a coastal road. In the 1960s, the docks of the Tsuyazaki fishing port were stone revetments constructed during the Taisho era (1912-1926).
1960s - 2000s	Watari Peninsula & Tsuyazaki tidal flat	③ ❹❺❻	Juvenile & Adult	Horseshoe crabs were frequently caught in fishing nets along the coast of the Watari Peninsula, including large ones which posed a problem. The horseshoe crabs caught in the nets were disposed of at Tsuyazaki fishing port, with their numbers being greater in the past. However, the numbers have gradually decreased over the years, and in the last decade (since approximately 2008), hardly any have been caught in the nets. Small horseshoe crabs were commonly spotted in the tidal flats in the old days but have never been seen on Mate Island. There used to be a seaweed farm on the north side of the tidal flats.

2.3.3 Spawning Survey Results: Number of Spawning Pairs between Sites and Changes in Spawning Numbers over time

T. tridentatus showed a significant habitat preference for spawning over the sampling period between 2005 and 2013 (Pearson's chi-square test, x^2 =273.9; *df* = 11; *p*<0.05). Of all spawning sites, Site 2 was the most concentrated area where 34 % of spawning pairs was found (75 pairs out of 223 pairs), and 22% at Site 1 (48 pairs out of 223 pairs). Thus, more than half of the pairs used Sites 1 and 2 for their spawning activities from 2005 to 2013 (*n*=223) (Figure 2.10).

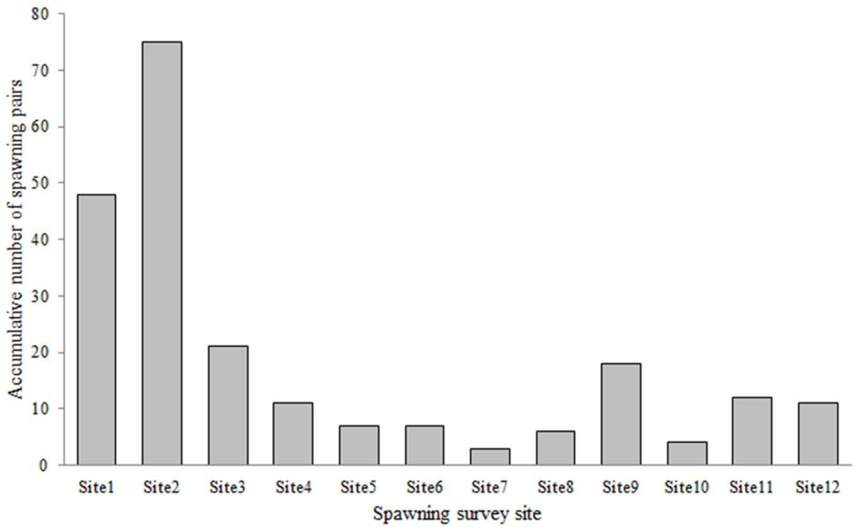

Figure 2.10 Accumulative number of spawning pairs at each survey site from 2005 to 2013. The locations of the survey sites are shown in Figure 2.4.

Figure 2.11 illustrates the number of observed *T. tridentatus* spawning pairs from 2005 to 2013. The highest recorded count was in 2005, with 83 pairs, after which there was a gradual decline each year. By 2009, the number of observed pairs had decreased to less than four. A significant decrease was observed after comparing the mean number of spawning pairs before and after 2009. The average number of pairs from 2009 to 2013 ($\bar{x}$=2.8±1.3) was significantly lower than the average from 2005 to 2008 ($\bar{x}$=49.5±25.3 pairs) (Mann-Whitney U test, *U* (4,5)= 0.0; *p*<

0.05), indicating a sharp decline in the number of *T. tridentatus* spawning pairs after 2009.

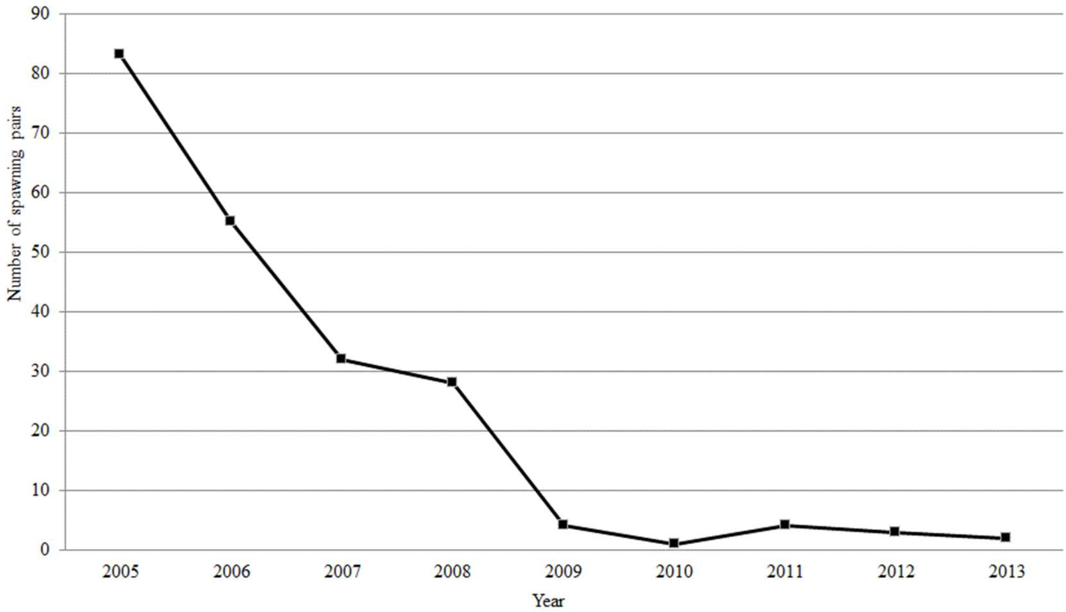

Figure 2.11 Changes in the number of *T. tridentatus* spawning pairs from 2005 to 2013 in Tsuyazaki, Fukuoka.

2.3.4 Overview of the Results

Figure 2.12 provides an overview of the status of the *T. tridentatus* population and the relationship between the spawning sandy beaches and the development of Tsuyazaki Cove from the post-war period to the present. The cove underwent significant development from the late 1960s to the 1970s, leading to a substantial reduction in the extent of sandy beaches. Furthermore, from the 1980s until the present, closure works were conducted around the bay mouth, exacerbating the decline of sandy beaches. The interviews revealed that the number of *T. tridentatus* sightings gradually decreased over time, and the species was rarely caught in fishing nets since around 2008. The field survey data showed a consistent decline in spawning pairs from 2005 to 2013.

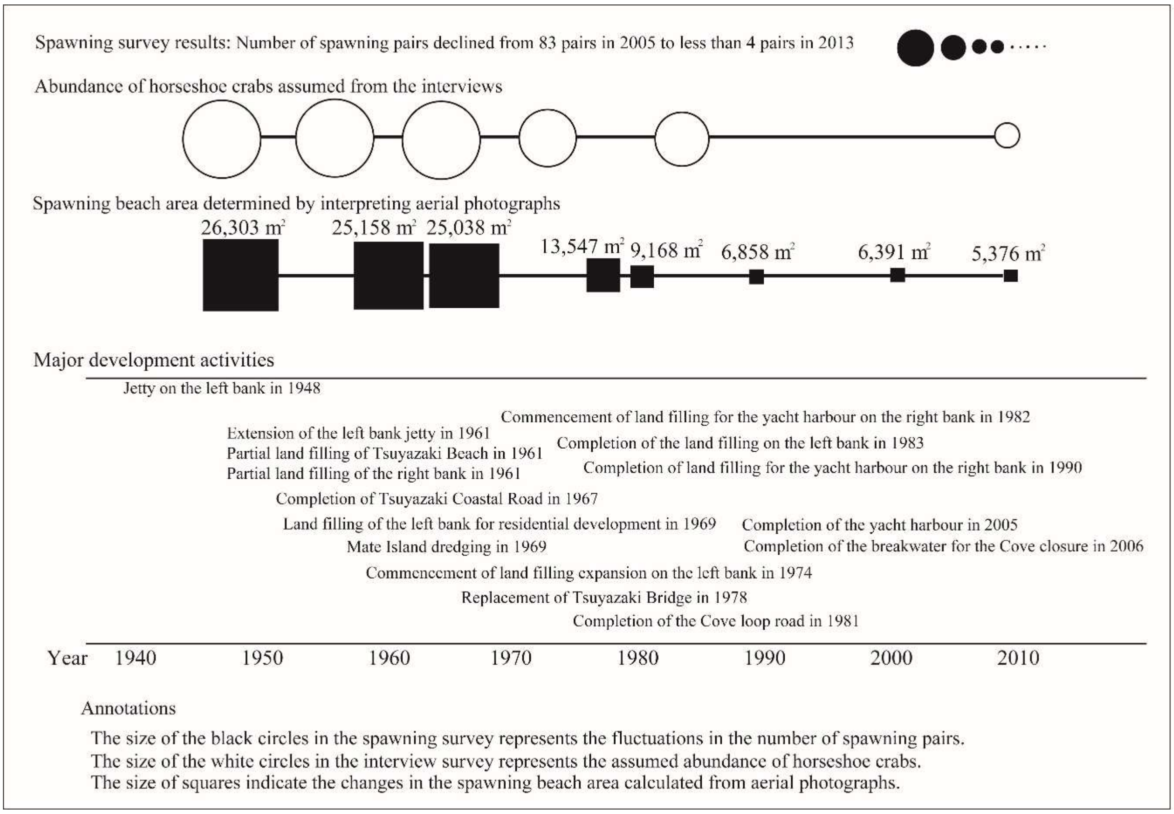

Figure 2.12 Overview of the results (Changes in spawning beach area associated with the bay mouth modification, *T. tridentatus* abundance assumed from the interview surveys, and actual spawning numbers in Tsuyazaki Cove).

2.4 Discussion

2.4.1 Changes in the Potential Spawning Sites from 1948 to 2010

The results of this chapter allow us to suggest that the decline of *T. tridentatus* in Tsuyazaki could be related to the loss of sand supply to spawning sites caused by the development of the bay mouth. This phenomenon was confirmed by the significant negative relationship between the amount of potential spawning sites and the development area from 1948 to 2010 (Figures 2.6 and 2.7).

Almost all *T. tridentatus* spawning habitats in Japan are located at river mouths where sediments are supplied from the rivers to form spawning grounds (Sekiguchi 1989). However, this is not the case in Tsuyazaki. The spawning sites of *T. tridentatus* in Tsuyazaki are situated on the surface of residual sand dunes from the Holocene epoch, deposited approximately ten thousand years ago (Hirowatari & Shimoyama, 1999) (Figure 2.2). A lack of sand supplies from small creeks, which were formed for salt farming during the seventeenth and eighteenth centuries, adjacent to Tsuyazaki Cove means that the coastal and tidal currents may play a significant role in supplying sand to the spawning sites. In addition, the presence of Watari Peninsula protruding on the southeast side of Tsuyazaki Cove provides a relative protection from waves generated by the westerlies (Figure 2.2). These geological and geographical features have provided a stable spawning habitat for *T. tridentatus* over a long period.

However, this subtle balance appears to have been devastated during the economic development and afterwards. The results suggest that the development of the bay mouth might significantly affect habitat degradation by changing the incoming and outgoing tidal current movements, which caused the reduction in the supply of sand from the open sea. During the 1940s, a jetty was already established on the left bank of the bay mouth to mitigate the inflow of drift sand from Tsuyazaki beach into the cove and to maintain a sufficient depth for the fishing port. Subsequent expansions of the jetty occurred in the 1960s. By the 1970s, the development around the fishing port was almost finished, leading to a depletion of sand supply from the left bank during that period (Figures 2.5, 2.6 and Table 2.1). The most substantial development, including coastal road construction, was implemented in the late 1970s. This construction involved land reclamation of approximately 10 meters towards the cove, leading to a significant loss of spawning beaches (Figure 2.6). Following this, the development of the right bank at the bay mouth started in the 1980s. The source of sand was also eventually reduced in 1990 due to the closure of the right bank. As a result, the accumulation of sand started at the corner of the yacht harbour, which otherwise could have flowed into the bay to provide spawning grounds for the horseshoe crabs. The sand deposition around the yacht harbour increased gradually between 1990 and 2010. After these developments, each sandy beach (i.e., spawning site) became completely isolated (Figure 2.6).

According to the spawning surveys conducted by Wakamiya (1989) in Fukuoka between 1981 and 1983, the sandy beach in Tsuyazaki was narrower compared to other areas such as Imazu and Kafuri, which led to the conclusion that the spawning grounds in Tsuyazaki were not as favourable for *T. tridentatus* as other areas. However, the result of the interview survey suggested that despite the unfavourable conditions noted by Wakamiya, many *T. tridentatus* were still present in Tsuyazaki until the 1980s (Figure 2.9 and Table 2.2). Even after the reclamation of the left bank was completed, the population of *T. tridentatus* was still maintained, most likely because sand continued to be supplied to the spawning grounds from the right bank of the bay mouth. However, the sand supply was reduced along with the development of the right bank, as described above. Therefore, the spawning sites in Tsuyazaki may have already been vulnerable in 2005 when the spawning survey started.

2.4.2 Deterioration of the Most Significant Spawning Sites resulting in the Decline of Spawning Pairs from 2005 to 2013

The spawning survey revealed that many *T. tridentatus* selected Sites 1 and 2 as their preferred locations, constituting more than half of the observed spawning activities (Figure 2.10). Wada et al. (2010) proposed a positive correlation between the median diameter of sediments and the abundance of spawning pairs in the same area. Although *T. tridentatus* in Tsuyazaki exhibited a wider range of grain size preferences for egg deposition compared to other spawning habitats in Japan (Wada et al., 2010; Iida et al., 2017), approximately 80% of the spawning activities occurred in sites characterised by fine to medium sands, including Sites 1 and 2 (Wada et al., 2010). Consequently, the conservation of these crucial spawning areas, particularly Sites 1 and 2, is imperative for the reproductive success and long-term survival of the local *T. tridentatus* population. However, there was a continuous decrease in the number of *T. tridentatus* spawning pairs, with a significant decline noted after 2009 (Figure 2.11). Fishermen in Tsuyazaki reported a reduction in *T. tridentatus* bycatch since 2008, which coincides with the sharp decrease in spawning numbers since 2009 (Table 2.2).

During the sampling period, Site 2, which had the highest number of spawning pairs, experienced beach erosion (Figure 2.8). This erosion likely impacted the shape of the spawning ground, potentially making it less suitable for nesting. Among the sites in Tsuyazaki, only Site 2 retains the supratidal vegetation zone, indicating that the original shape of the spawning ground has been partially preserved. Unfortunately, no comparative pictures are available for other sites, making direct comparisons difficult during sampling. However, even Site 2, considered the best spawning site in Tsuyazaki, showed degradation due to a lack of sand supply over the sampling period, suggesting that similar degradation may have also occurred at other sites. Replacing supratidal vegetation zones and high tide lines with seawalls in all other spawning sites has rendered them more vulnerable to erosion. The obstruction caused by the seawalls forces spawning adults to spawn directly beneath them (Itaya personal observation). According to Wakamiya (1989), the construction of seawalls may have caused the loss of the most suitable spawning grounds in Fukuoka. Similarly, in Tsuyazaki, too, many suitable spawning zones may have already been lost due to the construction of seawalls in most of the spawning sites.

Uchiyama (2001) reported that sandy beaches on tidal flats are in a state of dynamic equilibrium over the long term, with a constant cycle of erosion and deposition. However, the cumulative modifications at the bay mouth have disrupted the natural sand dynamics necessary for the stability of the spawning grounds. Sites 1 and 2 are located on the right bank of the bay mouth, where tidal currents from the open sea could flow smoothly into the cove, enabling a sequence of sandy beach formation (potential spawning sites) from the 1940s to 1960s. On the other hand, the left bank underwent direct land-filling starting in the early 1960s (Figure 2.5), resulting in the absence of continuous sedimentation since that time (Figure 2.6). Therefore, the right bank of the bay may have been crucial for the reproduction of the species during the sampling period (even though the spawning sites were already degraded to some extent when the spawning survey started as described). Nevertheless, the right bank was almost closed in 2005 by the completion of the yacht harbour (Figure 2.5). In addition, the construction of a breakwater in 2006 (Figure 2.5), aimed at protecting the yacht harbour from waves, potentially reduced the influx of sand into Tsuyazaki Cove, further degrading the spawning grounds (Figure 2.6). The sand deposition on the yacht harbour's western side, which started in 1990, indicates a disturbance in the natural sand transport routes that would typically supply sand to the spawning sites (Figure 2.6). The grain size composition of the sand deposited on the western side of the yacht harbour and Sites 1-3 is almost the same (Itaya unpublished data). It is, therefore, possible that artificial structures built after 2000 may have further degraded already fragile spawning grounds, leading to a sharp decline in spawning numbers from 2009 onwards.

Although the reasons behind the rapid decrease in spawning pairs since 2009 are not fully understood, in some cases, coastal developments lead to physical obstacles for the movement of *T. tridentatus* going out/into the bays and malfunction of the process of dispersal of the larvae due to changes in tidal movements (Maeda et al., 2000; Seino et al., 2000). In the surveys of *T. tridentatus* juveniles from 2003 to 2016 at Tsuyazaki Cove, approximately 80% of juveniles disappeared since 2009 when comparing the average number of juveniles per survey between 2003-2008 (2003-2008: $\bar{x}$=81.4±37.5, max.=145, min.=33, n=6) and 2009-2016 (2009-2016: $\bar{x}$=17.9±7.8, max.=31, min.=7, n=8) (Itaya unpublished data). This decrease in the juveniles and the decline of the spawning pairs during the same period in the area could be evidence of the impact of the construction of the yacht harbour and breakwater in the early 2000s.

Habitat size and development scale can be an issue when describing the impact on *T. tridentatus* local populations. In Sone tidal flat, which represents one of the largest habitats for *T. tridentatus* in Japan (Takeishi, 2016), the construction of an artificial island-type airport took place approximately three kilometres away from the spawning ground in 2006 (Hara et al., 2007), and further development projects are still ongoing (Takahashi, 2018). While the number of spawning pairs in the Sone tidal flat experienced a decline between 2006 and 2012, it has shown signs of recovery since 2013 (Hayashi, 2019). However, in Tsuyazaki, the number of spawning pairs has failed to rebound and remains on a continuous downward path (Figure 2.11). Despite these ongoing development activities, one potential explanation for the relatively abundant horseshoe crab population in the Sone tidal flat can be the larger habitat size it offers. With an area of approximately 517 hectares, ten times larger than Tsuyazaki Cove, the Sone tidal flat could provide a more favourable environment for horseshoe crabs. Conversely, Tsuyazaki Cove, which has a smaller habitat area, was already vulnerable during the current survey; It is assumed that in this situation, even small-scale developments, such as the construction of the yacht harbour and breakwater, can cause significant damage to local *T. tridentatus* populations.

Sandy beaches used as spawning grounds by *T. tridentatus* are formed under limited conditions requiring subtle balances. Therefore, it is vital to consider the ecological consequences of their habitat when changing these geomorphological features through developments (Seino et al. 2001). It seems likely that coastal developments with a poor understanding of ecological knowledge have caused the corruption of natural mechanisms to form the spawning beaches in the area. The cumulative development of the bay mouth and subsequent spawning beach reduction led to the gradual decline of *T. tridentatus* during the economic growth and afterwards.

Finally, the closure of the bay mouth in the early 2000s may have led to the inability of the population to sustain itself.

2.4.3 Future Issues in Determining the Factors responsible for the Decline of *T. tridentatus* in Tsuyazaki

In this chapter, the status of *T. tridentatus* since the 1940s was ascertained through interviews and the number of spawning pairs from 2005 to 2013 was investigated through field surveys in Tsuyazaki. Interviews confirmed that *T. tridentatus* in Tsuyazaki, which was thought to have been present in significant numbers in the past, had gradually declined and were rarely seen in the early 2000s (Table 2.2). The field surveys showed that the number of spawning pairs decreased annually since 2005, with a sharp drop to less than four pairs after 2009 (Figure 2.11). A reduction in spawning beaches due to bay mouth development was suggested as a contributing factor to the decline in the number of spawning pairs.

Despite the factors suggested in this chapter, adequate physical data on the habitat is lacking, making it challenging to identify the other potential causes of the species' decline. For example, the artificial modification of habitats may alter tidal currents, affecting the dispersal of hatchling juveniles (Maeda et al., 2000; Seino et al., 2000). Also, other factors, such as the impact of coastal development on their egg survival, may be responsible for the species' decline. To better understand the factors responsible for the species' decline and develop conservation strategies, it is desirable to gain a deeper understanding of the physical conditions of their habitat, including the effects of artificial modifications. Therefore, future research should focus on investigating these factors in greater detail.

2.4.4 The Value and Conservation of the *T. tridentatus* Population in Tsuyazaki

Monitoring the population and identifying the reasons for the decline are crucial research topics for preventing rare species from extinction (Miyashita & Fujita, 1996). However, the significant reduction in the *T. tridentatus* population in Tsuyazaki since 2009 can be described as a state of near-extinction, where the population size has declined to such a critical level that it cannot sustain itself through natural reproduction. With such a small population size, even without environmental changes, the population is at risk of extinction due to random fluctuations in population size, genetic drift, inbreeding, and other factors. Therefore, it is of utmost importance to conduct thorough investigations in the future to determine the factors that have contributed to the decline of *T. tridentatus*. Once these factors are identified, it is crucial to implement specific conservation measures that directly address them. One practical action that can be taken is restoring lost spawning grounds, creating more suitable habitats for *T. tridentatus*, and preventing the Tsuyazaki population from extinction. By gaining a deeper understanding of the underlying causes and taking targeted actions, the conservation and recovery of the *T. tridentatus* population in Tsuyazaki can be effectively pursued.

The population in Tsuyazaki is one of the genetic groups that make up the "Fukuoka Unit". While the genetic differentiation is statistically insignificant among the "Fukuoka Unit", the Tsuyazaki population has a slightly different genetic profile from other regions in Fukuoka (Nishida & Koike, 2009). Therefore, the extinction of the Tsuyazaki population could lead to a decline in *T. tridentatus* genetic diversity in Japan. As almost all of the *T. tridentatus* populations in Japan face the risk of extinction due to human activities (Sekiguchi, 1989; Sekiguchi, 1999), conserving their remaining habitats to protect their genetic diversity is imperative. Thus, from a phylogenetic conservation perspective, the genes of *T. tridentatus* in Tsuyazaki must be passed on to future generations.

The Tsuyazaki Tidal Flat and its surrounding agricultural area are designated an "Important Wetland for Biodiversity in Japan" by the Ministry of the Environment (M.E., n.d.). Furthermore, a part of these areas, along with the Munakata region, is recognised as a UNESCO World Cultural Heritage Site (UNESCO, 2017). These accomplishments deserve great recognition from a wide range of individuals. However, the condition of the natural environment in the area has deteriorated over the past decade or so. Until 2010, most of the agricultural areas were turned into concrete irrigation creeks, resulting in the rapid decline of another iconic endangered species, the Japanese rosy bitterling, *Rhodeus ocellatus kurumeus*, a rare freshwater fish known as a biological indicator of floodplain viability. Besides, more than half of the area of the previous salt farm became mega solar power plants in 2013. Thus, these environmentally essential components, namely the agricultural areas and the former site of the salt farm, which together form the wetland environment around the Tsuyazaki Tidal Flat, have suffered significant degradation. It is concerning that this degradation may result in alterations in freshwater and nutrient input into the tidal flat and lead to aridification in the area (Itaya et al., 2018). These significant changes in the natural environments call for a reassessment of the importance of preserving *T. tridentatus* in this area. The belief is that through diligent efforts to safeguard the survival of *T. tridentatus*, it becomes feasible to fulfil the requirements for "biologically important wetland" and "World Cultural Heritage". While development for the local industry is vital for the community, it may result in a significant environmental loss if *T. tridentatus* disappears due to excessive development. As a regional asset, the value of Tsuyazaki Cove, home to the endangered *T. tridentatus*, must be carefully considered, and measures should be taken to conserve it.

Although *T. tridentatus* is listed as critically endangered in Japan (Ito, 2014), these listings have no legal force, and there are no commitments imposed by their inclusion in Japan's Red Data List and Red Data Book and, no specific conservation strategies are applied for those species listed

(Hayama & Sekine, 2003). Therefore, to prevent the decline of *T. Tridentatus* populations in Japan, it is necessary to establish a legal framework for protecting species listed in the Red Data Book and incorporate scientific methods during environmental assessments.

2.5 Conclusion

This chapter aimed to investigate the habitat change process of *T. tridentatus* in Tsuyazaki Cove over time and compare it with interviews and monitoring of spawning pairs to gain insight into the factors behind the decline of *T. tridentatus*. The findings indicate that the modification of the bay mouth, leading to the obstruction of sediment transport routes, contributed to the decline of sandy beaches as a spawning ground. Coastal development made with a poor understanding of ecological knowledge and a lack of formal protection mechanisms for the species have caused the decline of the spawning sites and the corruption of natural processes previously involved in the formation of sandy beaches in the area.

This chapter demonstrated the effectiveness of tracing the history of habitat changes from the past to the present and matching the results to the current environment for understanding the situation prior to field surveys, particularly in cases where historical or physical habitat data for conservation target species is unavailable. This method is beneficial for conserving rare coastal species, such as horseshoe crabs, as it allows for an initial analysis of the causal relationship between population decline and coastal development at both spatial and temporal levels, which cannot be determined by field surveys alone.

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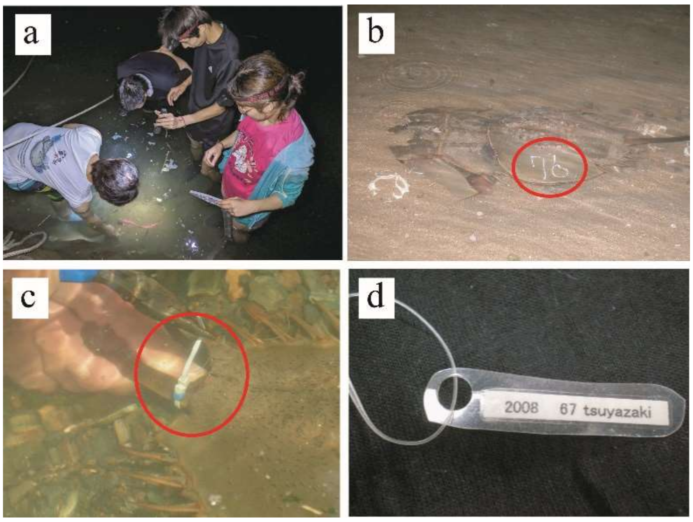
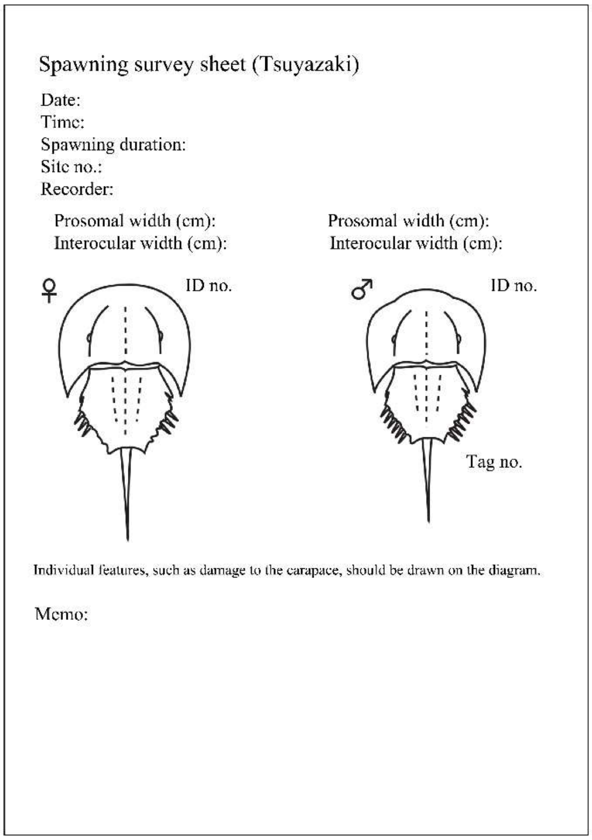
Appendices



Appendix 2.I Spawning survey and individual identification techniques in this chapter: a) The researcher applying ID numbers to a pair of *T. tridentatus*. b) Unique numbers marked on the sides of each horseshoe crab's carapaces using a crayon. c) Attachment of a tag with a unique number to the telson of male horseshoe crabs. d) A tag used for individual identification.


Spawning survey sheet (Tsuyazaki)

Date:
Time:
Spawning duration:
Site no.:
Recorder:

Prosomal width (cm):
Interocular width (cm):

♀ ID no.

Prosomal width (cm):
Interocular width (cm):

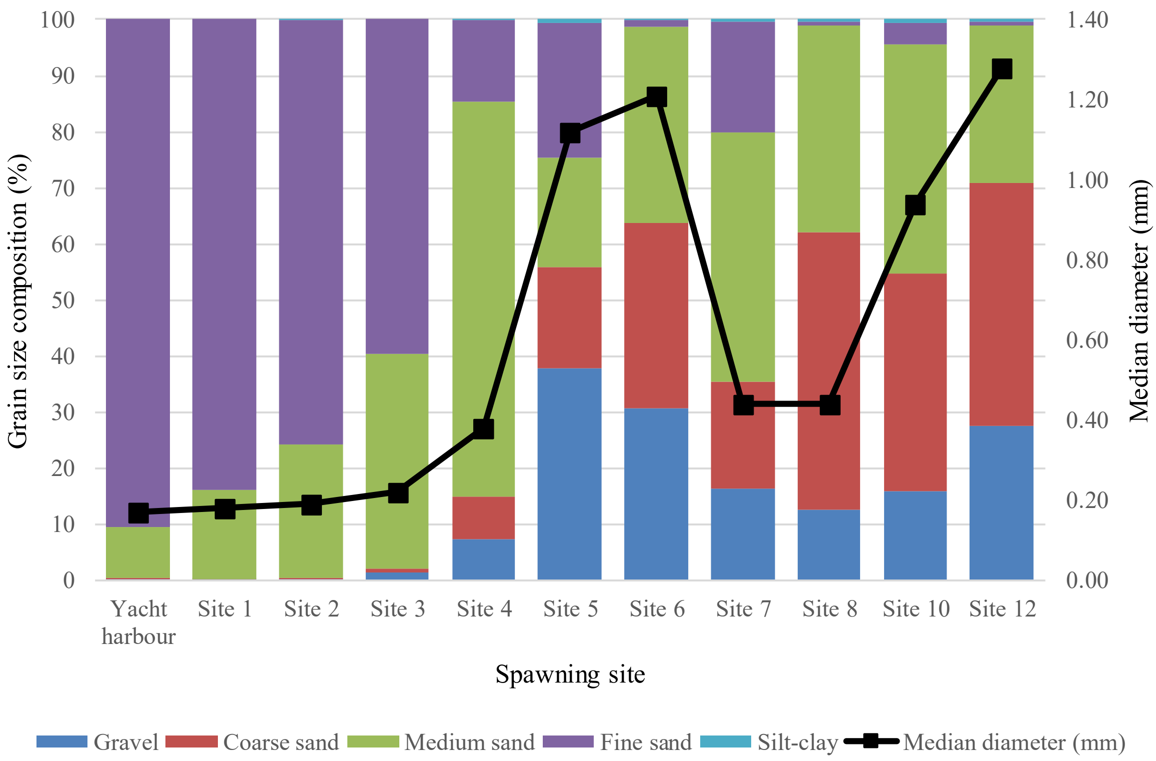
♂ ID no.

Tag no.

Individual features, such as damage to the carapace, should be drawn on the diagram.

Memo:

Appendix 2.II Spawning survey sheet used in the study of this chapter.



Appendix 2.III Comparison of grain size compositions and median diameters between spawning sites and sand deposited near the yacht harbour. Sediment similarities indicate the retention of sand at the harbour instead of its transport to Sites 1-3. No sand sampling at Site 9 due to the absence of spawning ground. Sampling was conducted in 2018.

Chapter 3

Evaluation of the Spawning Site Selection and Nesting Patterns of *Tachypleus tridentatus* in the Modified Spawning Habitat

Abstract The spawning site selection patterns of *Tachypleus tridentatus* were investigated in relation to beach elevation, slope, and grain size to assess the underlying impacts of coastal development in Tsuyazaki, Fukuoka, Japan. The results indicated that most spawning activities occurred within a narrow range of beach elevation (T.P. +0.60 to +0.89 m) and slope (6.00° to 6.99°). However, some spawning activities were observed in areas characterised by lower and less steep spawning sites, where the original shape of the spawning ground had been modified due to coastal development. This suggests that the species may have limited options for depositing their eggs, as they are confined to a small, fragmented beach area. These findings highlight the preference of *T. tridentatus* for spawning sites that exhibit relatively well-preserved beach shapes, offering favourable elevation, slope, and sediment characteristics. The potential underlying reasons for these observations are discussed, and management implications are proposed.

Keywords: coastal management, endangered species, habitat restoration, habitat selection

3.1 Introduction

The spawning of *Tachypleus tridentatus* takes place mostly from late June to August in Japan. During this time, the species deposits its eggs on the gently sloping sandy beaches near the high tide line (Sekiguchi, 1999). The decline of *T. tridentatus* in Japan is primarily attributed to human activities, resulting in the loss of tidal flats and spawning beaches. Habitat loss, particularly due to coastal land reclamation for economic development, such as industrial and agricultural expansions, has led to a decline in *T. tridentatus* populations in Japan (Sekiguchi, 1989; Sekiguchi, 1999; Akbar John et al., 2018). The species faces an extinction threat, necessitating urgent conservation measures to ensure its continued existence (Seino et al., 2000). In particular, the conservation and restoration of sandy beaches utilised as spawning grounds by *T. tridentatus* assume great importance, considering the species' ongoing rapid population decline (Wada et al., 2010).

While there are still uncertainties regarding the nesting preferences of horseshoe crabs, some studies suggest that beach elevations and sediment characteristics play a crucial role in egg development (e.g., Penn & Brockmann, 1994; Hsieh & Chen, 2009). In addition, Botton et al. (1988) observed that beach revetments causing sand erosion rendered areas unsuitable for spawning in the Atlantic horseshoe crab (*Limulus polyphemus*). In Tsuyazaki, the spawning population of *T. tridentatus* has substantially reduced, primarily caused by coastal development activities, leading to the degradation and loss of critical spawning sites (Itaya et al., 2019). Therefore, it is imperative to investigate the causal factors of this decline, mainly related to coastal developments, to conserve this rare species effectively. This chapter served as a preliminary investigation aimed at exploring the spawning site selection of *T. tridentatus* in relation to the physical characteristics of the known spawning beach and assessing the fundamental impact of coastal development in Tsuyazaki, Fukuoka, Japan.

3.2 Methods and Materials

3.2.1 Study Site

The field investigation was conducted at Tsuyazaki Cove in Fukuoka (Figure 3.1). The area encompassing Sites 1, 2a, 2b, 3, and 4, near the bay mouth, is considered a crucial spawning ground where approximately 70% of spawning activities were observed between 2005 and 2008 (Wada et al., 2010). However, these critical spawning sites have degraded due to coastal development, leading to a rapid decline in the spawning pairs (Itaya et al., 2019).

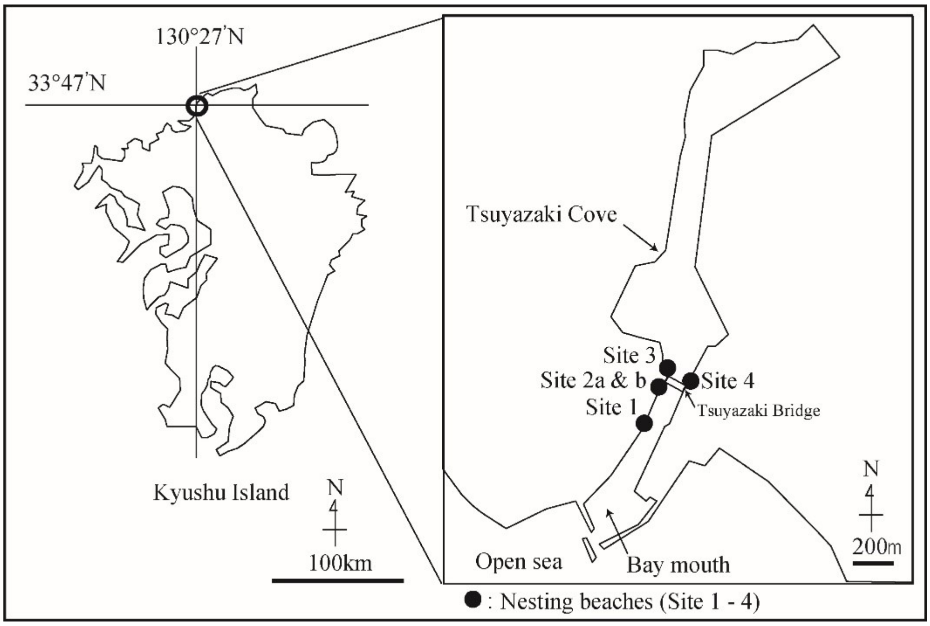


Figure 3.1 Study site.

3.2.2 General Methods and Analysis

Sites 1 - 4 were surveyed to identify nesting locations during high flood tides between July and August 2018. Each survey spanned from two hours before the highest tide to one hour after, encompassing morning and nighttime high tides. Nest locations were marked with plastic stakes positioned adjacent to the breeding pairs. Subsequently, the coordinates of each nest were recorded during low tide periods using the Real-Time Kinematic-Global Navigation Satellite System (RTK-GNSS: Trimble R4 GNSS, Nikon-Trimble Co., Ltd.). Digital elevation maps were generated for each spawning site using ArcGIS ver. 10.6.1. These maps were then overlaid with the recorded nest locations to estimate each nest's elevation and slope. The beach elevation based on T.P. (Tokyo Peil: the average sea level in Tokyo Bay) as 0 m, the standard for expressing elevations in Japan, was used in this chapter. Additionally, sediment samples were collected for grain analysis at each site (For more details on the grain size analysis, see the method section of Chapter 5). The species' preferences for the nesting environment were speculated using these parameters (i.e., nesting elevation, slope and sediment types). Principal Component Analysis (PCA) was conducted using R version i386 3.4.4 to assess beach elevation and slope contributions to nesting patterns.

3.3 Results and Discussion

The spawning site characteristics are summarised in Table 3.1. A total of 23 nests were found, with 8 nests at Site 2a, 5 at Site 2b, 9 at Site 3, and 1 at Site 4. No nest was found at Site 1 (Table 3.1 and Figure 3.2). The reason for no spawning at Site 1 in this chapter is probably due to the rapid decline of the local population, as described in Chapter 2. Sites 1 and 2 are the most common spawning sites (Wada et al., 2010; Itaya et al., 2019). However, with the drastic decrease in spawning numbers in Tsuyazaki, spawning is more likely to occur less than intended. Therefore, the fact that no spawning occurred during the survey in this chapter does not mean that Site 1 is unsuitable for spawning (See more details for this discussion in Chapter 5).

The spawning sites exhibited sediment characteristics with a medium diameter range of 0.18 to 0.34 mm and a size-sorting range of 0.51 to 1.06 φ. Sites 2a and 3, characterised by fine sand with a medium diameter range of 0.19 to 0.22 mm and a grain size-sorting range of 0.59 to 0.73 φ, showed the highest frequency of spawning activities. Sites 1 and 4, which had the lowest and second lowest occurrence of nesting, consisted of fine and medium sand with a medium diameter ranging from 0.18 to 0.34 mm (Table 3.1). Previous studies in Japan have reported *T. tridentatus* depositing eggs across a wide range of grain sizes, ranging from 0.09 mm (Iida et al., 2017) to 5.00 mm (Wada et al., 2010). Therefore, the sediment properties observed at all the sites fall within an acceptable range for spawning. Consequently, sediment differences may not be the primary determining factor influencing the selection of spawning grounds in the study site.

Table 3.1 Summary of nest count, sediment characteristics, presence of supratidal zone, and location of seawalls at each spawning site.

Site	Number of nests	Sediment			Supratidal zone	Sea wall †
		Grain size classification	Median diameter (mm)	Grain size sorting (φ)		
1	0	FS	0.18	0.51	Disappeared	Below HTL
2 a	8	FS	0.19	0.59	Existing	Above HTL
2 b	5	MS	0.31	0.71	Disappeared	Below HTL
3	9	FS	0.22	0.73	Disappeared	Below HTL
4	1	MS	0.34	1.06	Disappeared	Below HTL

Notes: †The location of seawalls. HTL refers to the high tide line. MS, medium sand; FS, fine sand.

Figure 3.2 Nest locations at Sites 2a, 2b, 3, and 4.

The highest spawning activity occurred at Sites 2a and 3, with nesting elevations ranging from T.P.+0.60 to T.P.+0.89 m and slopes between 6.00° and 6.99° (Figures 3.3). Nesting elevation varied from T.P.+0.09 to T.P.+0.90 m: $\bar{x}$=T.P.+0.83±0.04m (n=8) at Site 2a, $\bar{x}$=T.P.+0.12±0.03m (n=5) at Site 2b, $\bar{x}$=T.P.+0.70±0.04m (n=9) at Site 3, and $\bar{x}$=T.P. +0.85m (n=1) at Site 4. 78% of the nests were found at higher elevations ranging from T.P.+0.61 to T.P.+0.90 m at Sites 2a, 3, and 4. In contrast, Site 2b had nests at lower elevations between T.P.+0.09 and T.P.+0.17 m, accounting for 22% of the total nests (Figure 3.3a). The nesting slope ranged from 3.29° to 8.26°, with values of $\bar{x}$=5.41±1.33° (n=8) at Site 2a, $\bar{x}$=4.63±0.55° (n=5) at Site 2b, $\bar{x}$=7.55±0.66° (n=9) at Site 3, and 6.79° (n=1) at Site 4 (Figure 3.3b). The principal component analysis revealed that beach elevation significantly contributed to nesting at Sites 2a and 4, explaining 69.3% of the observed variation. In contrast, the nests at Site 3 showed a stronger association with the beach slope. Neither beach elevation nor slope influenced nesting at Site 2b (Figure 3.4). These findings suggest that *T. tridentatus* may prefer beaches with relatively high elevations and gradual slopes for spawning. However, some nests were also observed on a sandy beach with lower elevations and slopes.

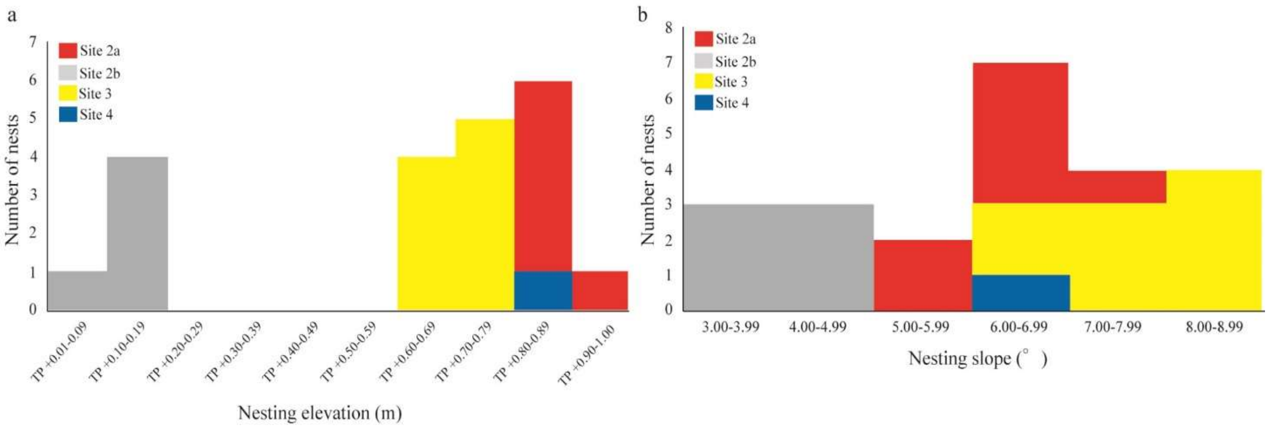


Figure 3.3 The relationship between a) beach elevation, b) slope and the number of nests.

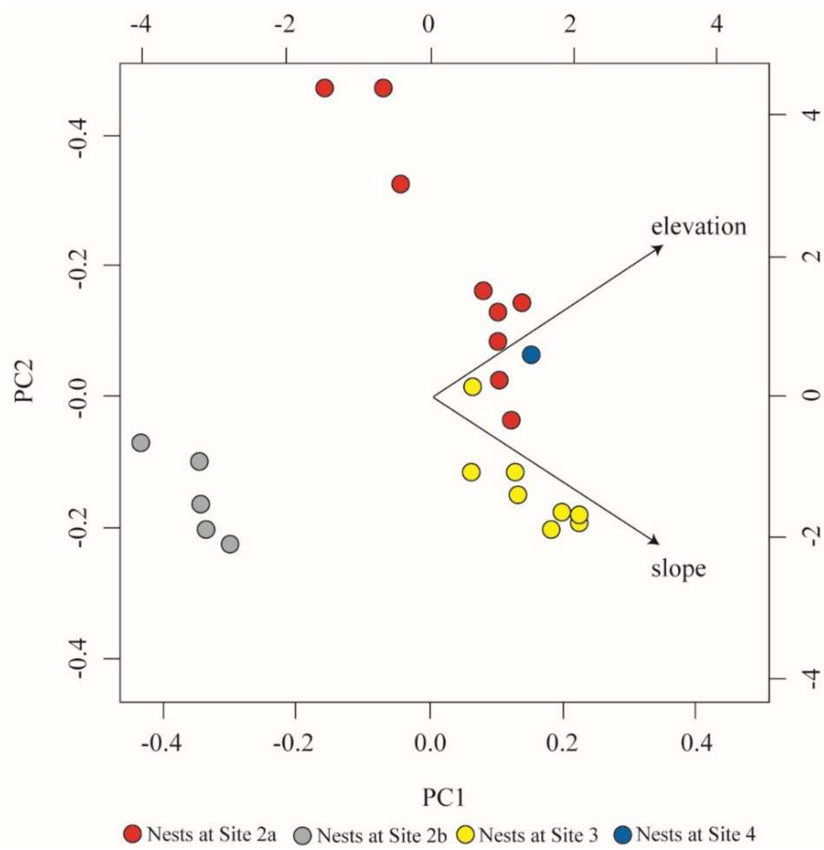


Figure 3.4 Principal Component Analysis (PCA) showing the contribution of beach elevation and slope in relation to nest location.

During surveys of spawning grounds in Fukuoka from 1980 to 1983, Wakamiya (1989) speculated that the most suitable spawning grounds for *T. tridentatus* had already been destroyed due to the construction of seawalls below the high tide line. The presence of seawalls and bulkheads within the intertidal zone significantly reduces the available spawning habitat for horseshoe crabs, as they typically lay their eggs on gently sloping sandy beaches near the high tide line (Botton et al., 1988; Jackson et al., 2015). In Tsuyazaki, the supratidal zones and high tide lines have been extensively replaced with seawalls, except for Site 2a (Table 3.1). Adult horseshoe crabs were observed searching for suitable spawning areas along these seawalls in the affected sites. In contrast, such behaviour was not observed at Site 2a, where no seawalls were present below the high tide line (Itaya personal observation) (Figure 3.2). Although there was no considerable difference in the number of nests between Site 2a (8 nests), Site 3 (9 nests), and Site 2b (5 nests), their spatial distribution varied across these sites (Site 4 is not discussed in detail due to the small sample size of only one nest). The nests were dispersed widely at Site 2a, while at Site 3 and Site 2b, spawning activities were confined to specific areas near the seawalls (Figure 3.2). Site 2a displayed a relatively well-preserved original shape of the spawning ground, evident from a supratidal vegetation zone above the high tide line. In contrast, the original beach shapes at Sites 2b and 3 were extensively degraded due to landfills that altered the high tide line (Table 3.1 and Figure 3.2), suggesting that suitable spawning zones are limited at degraded sites.

The nests at Site 2a were observed to be located at the highest elevation (Figure 3.3), indicating that *T. tridentatus* prefers higher positions on sandy beaches with sufficient beach height for spawning (Figure 3.4). In contrast, the beach slope appeared to influence spawning site selection at Site 3, where the construction of a sea wall below the high tide line has limited the availability of suitable beach elevation for spawning (Figure 3.4). Nests at Site 2b were located at lower elevations and gentler slopes than previously known (Figures 3.3 and 3.4). *T. tridentatus* tends to nest at lower elevations where the most suitable spawning zones have disappeared due to coastal development (Sekiguchi, 1999); This pattern was also found at Site 2b indicating that the degradation of the original beach shape has led to a scarcity of suitable spawning zone.

The availability of oxygen is crucial for egg development in the nest (Sekiguchi, 1999; Hsieh & Chen, 2009). The egg development rate in the nests at lower elevations may be limited due to reduced exposure to air during low tides. Conducting additional measurements, such as evaluating the egg development rate at various beach elevations and estimating suitable spawning zones, would significantly enhance the investigation into the species' spawning site selection. This comprehensive approach would yield valuable insights into the species' preferences, behaviours, and the potential impact of coastal development on the availability of suitable spawning sites.

3.4 Conclusion

In conclusion, it seems that the alteration of beach shapes due to landfills has significantly impacted the availability and quality of suitable spawning grounds for *T. tridentatus*. Site 2a, with its well-preserved original spawning ground shape, stands out as a potential refuge providing a diverse range of nesting elevations. Therefore, conservation efforts should focus on protecting and restoring natural beach features to ensure the continued availability of suitable spawning habitats for this critically endangered species.

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Chapter 4

Effect of Intertidal Elevation on Survival Rate of *Tachypleus tridentatus* Eggs

Abstract Physical factors affecting the survival of *Tachypleus tridentatus* eggs were investigated by translocating their eggs between the high intertidal zone and the low intertidal zone of a known spawning site. The mean egg survival rates per day were highest in the mid intertidal zone ($\bar{x}$=45.1±25.4%) and the lowest in the low intertidal zone ($\bar{x}$=13.3±27.6%). Differences in the beach elevation, air exposure time, and water content of the spawning ground were significant factors determining the egg survival rates. Excessive or insufficient air exposure time resulted in inadequate water content at higher and lower intertidal zones and could reduce egg survival. On the other hand, moderate saturation and dehydration were repeated with each tidal movement in the mid intertidal zone. This dynamic is considered one of the crucial factors for the survival of eggs and is considered optimal for spawning. Therefore, protecting the mid intertidal zone is imperative for maximising the egg survival rate in Tsuyazaki Cove, where almost all suitable nesting sites have disappeared due to coastal development. By safeguarding these suitable spawning zones and restoring other sites, significant contributions can be made to future management and conservation efforts. The study of this chapter further proposes that translocating eggs from marginal areas to suitable spawning zones could serve as a potential strategy for recovering this globally endangered species.

Keywords: air exposure time, globally endangered species, intertidal elevation, intertidal zone, suitable spawning site, translocated horseshoe crab eggs

4.1 Introduction

Conservation and restoration of the spawning grounds of *Tachypleus tridentatus* are one of the most important issues for the survival of this species, whose population is declining significantly (Hsieh & Chen, 2009; Wada et al., 2010; Itaya et al., 2019b). *T. tridentatus* lay their eggs only on the calm sandy beaches of inner bays. The species spawns in the intertidal zone between the mean higher high water (MHHW) and the mean high water neap (MHWN) (Sekiguchi, 1999). However, the species that have lost suitable spawning sites due to coastal development may lay eggs at lower elevations than the MHWN (Sekiguchi, 1999). In Tsuyazaki Cove, the number of *T. tridentatus* spawning pairs has decreased remarkably. One of the causes is the decrease of sandy areas suitable for spawning due to coastal development (Itaya et al., 2019b; Itaya et al., 2022). Nesting at lower elevations than the MHWN was also found in Tsuyazaki (Itaya et al., 2019a). Some studies point out that the survival rate of eggs in such an unfavourable environment is low (Penn & Brockman, 1994; Vasquez et al., 2015). On the other hand, there are no field-based experiments on the effects of different elevations from high to low tide levels on *T. tridentatus* eggs. Therefore, there is a lack of quantitative data on the causal relationships between egg survival and coastal development impacts for the restoration and conservation of *T. tridentatus* spawning grounds.

In this chapter, *T. tridentatus* eggs were translocated at different intertidal elevations. Then, the relationship between the egg survival rate and environmental features was identified to find the most suitable nesting zone for egg development. Finally, the conservation measures for the species were suggested based on the findings.

4.2 Methods and Materials

4.2.1 Study Site

The study of this chapter was conducted at Tsuyazaki Cove in Fukuoka (Figure 4.1a). The spawning of *T. tridentatus* has been observed in small sandy areas along Tsuyazaki Cove, with approximately 70% of the spawning occurring near sites close to Tsuyazaki Bridge (Wada et al., 2010). In Tsuyazaki Cove, the spawning grounds have experienced a significant decline due to coastal development after World War II, contributing to a rapid decrease in the number of spawning pairs. This intensive development has resulted in the removal of nearly all suitable sandy beaches used for spawning in Tsuyazaki Cove (Itaya et al., 2019b; Itaya et al., 2022).

4.2.2 Egg Translocation Experiment and Analysis

T. tridentatus eggs were translocated to test the hypothesis that the egg survival rate is affected by the physical parameters at different elevations of the intertidal zone within the spawning ground. The high and low tide zones in the translocation site were decided as follows. First, the site's elevation and latitude/longitude data were obtained using the Real-Time Kinematic-Global Navigation Satellite System (RTK-GNSS: Trimble R4 GNSS, Nikon-Trimble Co., Ltd.). Then, these data were taken into ArcGIS (ArcMap version 10.8, ESRI Japan Co., Ltd.) to create a digital elevation map of the site. Next, changes in tidal levels over the study period were measured using a portable water depth meter (COMPACT-TD ATD-HR, JFE Advantech Co., Ltd.). Then, tidal ranges were calculated based on the definition of the Japan Coast Guard (2022) as follows: MHHW (Mean Higher High Water) for the average highest tides, MHW (Mean High Water) for the average high tides, MHWN (Mean High Water Neap) for the average neap high tides, MLWN (Mean Low Water Neap) for the average neap low tides. In this chapter, these tidal ranges were defined for consistency of terminology: MHHW as the high intertidal zone, MHW as the upper-mid intertidal zone, MHWN as the lower-mid intertidal zone, and MLWN as the low intertidal zone (For the diagram of the tidal range, see the method section of Chapter 5). Finally, the position of the high intertidal zone to the low intertidal zone within the translocation site was determined by overlaying MHHW/MHWN and the digital map. In addition, to confirm the consistency of the elevation map, aerial photographs of the MHHW and the MHWN at the translocation site were taken using a drone (Spark, Dji Co., Ltd.).

T. tridentatus spawning activities take place at high tide during summer. A female crab digs holes in a sandy beach and lays eggs in the holes. Then a male crab releases sperm there to fertilise the eggs (Sekiguchi, 1999). The eggs laid by the same spawning pair on 3 July 2019 on the same beach in the study site were collected for translocation on 4 July 2019. Since *T. tridentatus* has external fertilisation, it was assumed that fertilised eggs were distributed homogeneously in all translocated eggs. Two transect lines, transect A (A1 to A4) and transect B (B1 to B4), were placed on the sandy beach of the translocation site. Four translocation points (A1 to A4, B1 to B4) were set on each transect line. The translocation points were located in the high intertidal zone (near the MHHW), upper-mid intertidal zone (near the MHW), lower-mid intertidal zone (near the MHWN), and low intertidal zone (near the MLWN). Totally 100 eggs were translocated at each point of eight locations (Figure 4.1b). For translocating eggs, a plastic hydroponic mesh pot (600 mL) with a nylon net (mesh size 1 mm) spread out inside the pot was used to prevent egg loss and human disturbance as there are houses nearby, and many people use the site. The translocated eggs were placed in the pots together with the sand collected from the study site. The eggs were slightly separated from each other to prevent infection within the pot (Figure 4.2). Then, the hydroponic pots were buried 15 cm below the surface of each translocation point on the transects. The translocated eggs were buried at a depth at which horseshoe crabs lay eggs in the wild, as Maeda et al. (2000) and Chen et al. (2004) described.

T. tridentatus eggs become "rotating eggs (late embryos)" as they approach hatching (Sekiguchi, 1999). After the stage of "rotating eggs", the dispersion of the hatched larvae occurs, making it difficult to count the surviving eggs accurately. Therefore, the eggs were observed during the period from eggs (stage 1) to late embryos or "rotating eggs" (stage 21) based on the classification by Sekiguchi (1999). There are some detailed reports on the development of *T. tridentatus* eggs in captivity (Sugita & Sekiguchi, 1981; Sugita et al., 1985; Sekiguchi, 1999). However, unlike in captivity, it was impossible to observe the detailed developmental stages of horseshoe crab eggs in the field. Therefore, the eggs were not categorised into detailed developmental stages; instead, it was determined whether they were alive or dead. Infected, decayed, and broken eggs were judged as dead eggs. During the inspection, the eggs at each point were quickly transferred to a plastic tray full of seawater to determine how many eggs were dead or alive. After counting the surviving eggs and photographing them, they were buried in their original locations. Of the two transect lines, transect B was inspected at about two-week intervals until August 21, 2019, to observe the survival status of the eggs. The final surviving eggs at transect A were counted without digging until August 21, 2019.

The survival rate of the translocated eggs was calculated as:

Egg survival rate (%) = Number of eggs survived /Number of eggs translocated ×100. (4.1)

Since each hydroponic pot contained 100 eggs, the survival rate of eggs was equal to the number of surviving eggs. The survival rate of eggs at each translocation site during the experimental period was analysed using only transect B. For the comparison of the egg survival rates at each translocation point from B1 to B4, the mean survival rate per day was calculated after interpolating the data using a linear regression approximation formula. A significant test of the mean survival of eggs per day between the translocation points was performed by one-way ANOVA. When $p < 0.05$, the difference was considered significant. If a significant difference was evident, then the Tukey-Kramer procedure was used to examine where the significant difference lay.

4.2.3 Measurement and Analysis of Physical Parameters

To help understand the relationship between egg viability and the physical characteristics of each of the translocation points, salinity, nest temperature, air exposure time, water content, and sediment types were measured and analysed. Seawater near the translocation site was measured for salinity every 30 minutes over the study period (*n*=2001) with an underwater electrical conductivity measurement data logger (HOBO U24, Onset Co., Ltd.). The nest temperature was measured every 30 minutes over the study period (*n*=2065 for each point) by burying an underwater temperature measurement data logger (HOBO U24, Onset Co., Ltd.) 15 cm below the ground surface at each translocation point. The air exposure time of the translocation points was estimated from the differences in the elevation of each point and the tide levels for all the time zones of the study period (*n*=42 for each point). For the water content, the flood tide from August 17 to 18, 2019, was selected, and about 100 g of sediment 15 cm below the ground surface at each translocation point was collected every hour. Then, the water content ratio in the sediment was calculated as the difference between wet weight and dry weight:

$$W=100(W_w/W_s), \qquad (4.2)$$

where, W = water content, Ww = weight of water contained in sediment, Ws = weight of dried sediment. Sediment samplings were not possible when the sand was immersed about 10 cm or more below the surface water because the sediment mixed with seawater. Therefore, the sediment samples for the water content were collected during low tides (*n*=25 at B1, *n*=21 at B2, *n*=13 at B3, *n*=7 at B4, respectively). About 100 g of sediment 15 cm below the ground surface was collected at each translocation point for the grain size analysis. Seven types of sieves with a mesh size of 0.064 mm to 4.00 mm were used for the grain size analysis. Dried sediment samples were passed through each sieve, and the grain size composition was determined from the weight of the particles remaining on each sieve and the ratio of the total weight. In addition, as representative values of the sediment at each translocation point, the median diameter and the grain size-sorting were calculated from the grain size distribution, and the silt-clay content was calculated from the ratio of silt-clay to the total weight (For more details on the grain size analysis, see the method section of Chapter 5). The display of physical data corresponding to each translocation point is described as B1 to B4 in this chapter (Figure 4.1b).

4.2.4 Analysis of the Relationship between Egg Viability and Physical Parameters

Generalised linear models (GLMs) were applied to find out the relationship between the egg survival rate per day and the physical parameters using R version 3.4.4. In these models, the elevation, the mean salinity, the mean nest temperature, the mean air exposure time, the mean water content, the median diameter, the grain size-sorting, and the silt-clay content were utilised as dependent variables (binomial distribution), and the mean egg survival rate per day was employed as a predictor variable. The square value of each physical parameter was also used as a predictor variable (ordinary least squares). The models with a significant p-value ($p < 0.05$) were regarded as robust models, and thus, these selected physical parameters were employed to estimate the mean egg survival rate per day.

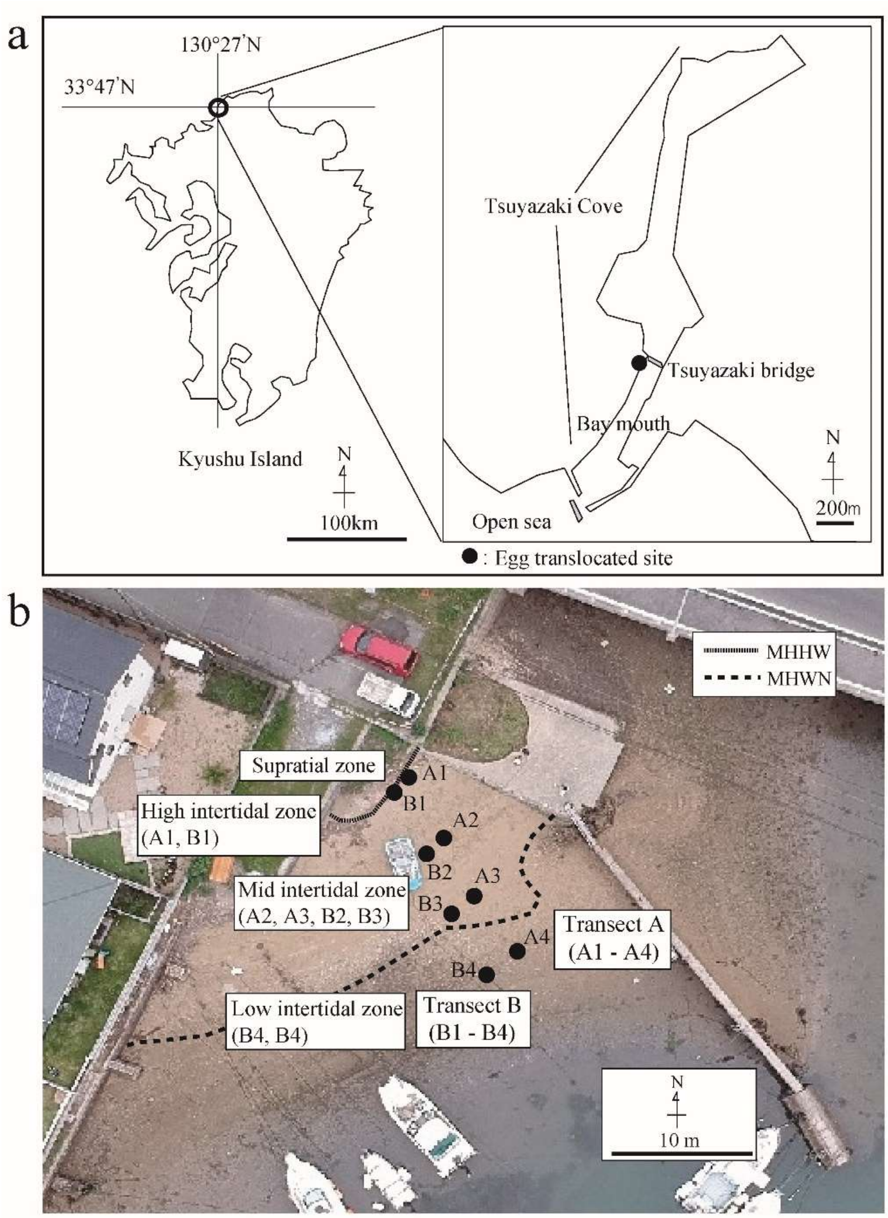


Figure 4.1 a) Location of the study site in Tsuyazaki, Fukuoka, Japan. The dots indicate the sites where *T. tridentatus* eggs were translocated in the study of this chapter. b) The translocation site of *T. tridentatus* eggs in Tsuyazaki, Fukuoka, Japan. The mean higher high water (MHHW) and the mean high water neap (MHWN) are shown in the broken lines. The dots indicate the translocation points (A1-A4 and B1-B4).

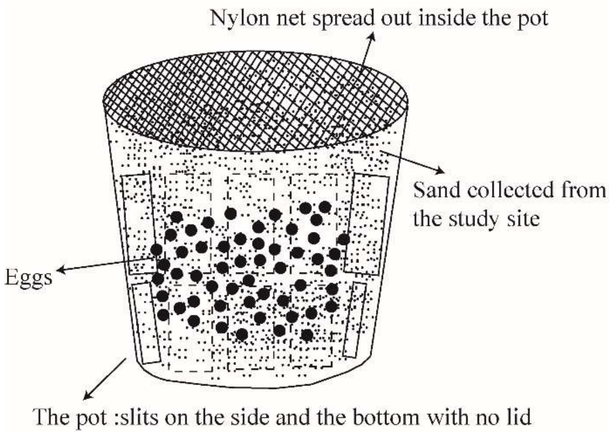


Figure 4.2 A diagram of a hydroponic mesh pot (600 mL) used for translocating the eggs. A nylon net (mesh size 1 mm) was placed inside the pot to prevent egg loss. Totally 100 horseshoe crab eggs were placed in each pot together with local sand, and they were buried 15 cm below the ground surface, which is the depth at which the horseshoe crab spawns in the wild.

4.3 Results

4.3.1 Egg Survival Rate

"Rotating eggs" were found only in the mid intertidal zone at both transects A and B (Figure 4.3). On transect A, the eggs in the mid intertidal zone became "rotating eggs" on August 21, 2019, 49 days after the translocation, but the survival rate was only 1%. On the other hand, 10% of eggs survived as "rotating eggs" in the mid intertidal zone on transect B. Although "rotating eggs" were confirmed on transect A, there was not enough data to analyse. Therefore, only the transect B data was used to analyse the following results.

On transect B, "rotating eggs" were found only in B3, the mid intertidal zone. These "rotating eggs" were first confirmed 44 days (August 16, 2019) after the translocation. By the 49th day (August 21, 2019), 10% of the "rotating eggs" in B3 were still alive. The lowest survival rate was found in the low intertidal zone (B4), where all eggs died within 13 days after the translocation

(July 16, 2019). In other intertidal elevations, more than 50% of eggs survived until day 13 (July 16, 2019), but in the high intertidal zone (B1), eggs had died out by day 28 (July 31, 2019). In the mid intertidal zone, the survival rate of eggs on the 28th day (July 31, 2019) was 18% for B2 and 40% for B3. However, on the 44th day (August 16, 2019), the eggs all died in B2 and only survived in B3 (Figure 4.4a).

The mean survival rate of eggs per day was the highest in the mid intertidal zone and the lowest in the low intertidal zone ($\bar{x}$=27.8±33.9% at B1, $\bar{x}$=34.1±30.8% at B2, $\bar{x}$=45.1±25.4% at B3, $\bar{x}$=13.3±27.6% at B4). A significant difference in the mean survival rate of eggs per day among the translocation points was demonstrated by one-way ANOVA (F=9.87; df=3,192; p=4.36^{-6}). Multiple comparisons based on the Tukey-Kramer test revealed that the survival rate of eggs was significantly lower in the low tide zone ($p<0.05$) (Figure 4.4b).

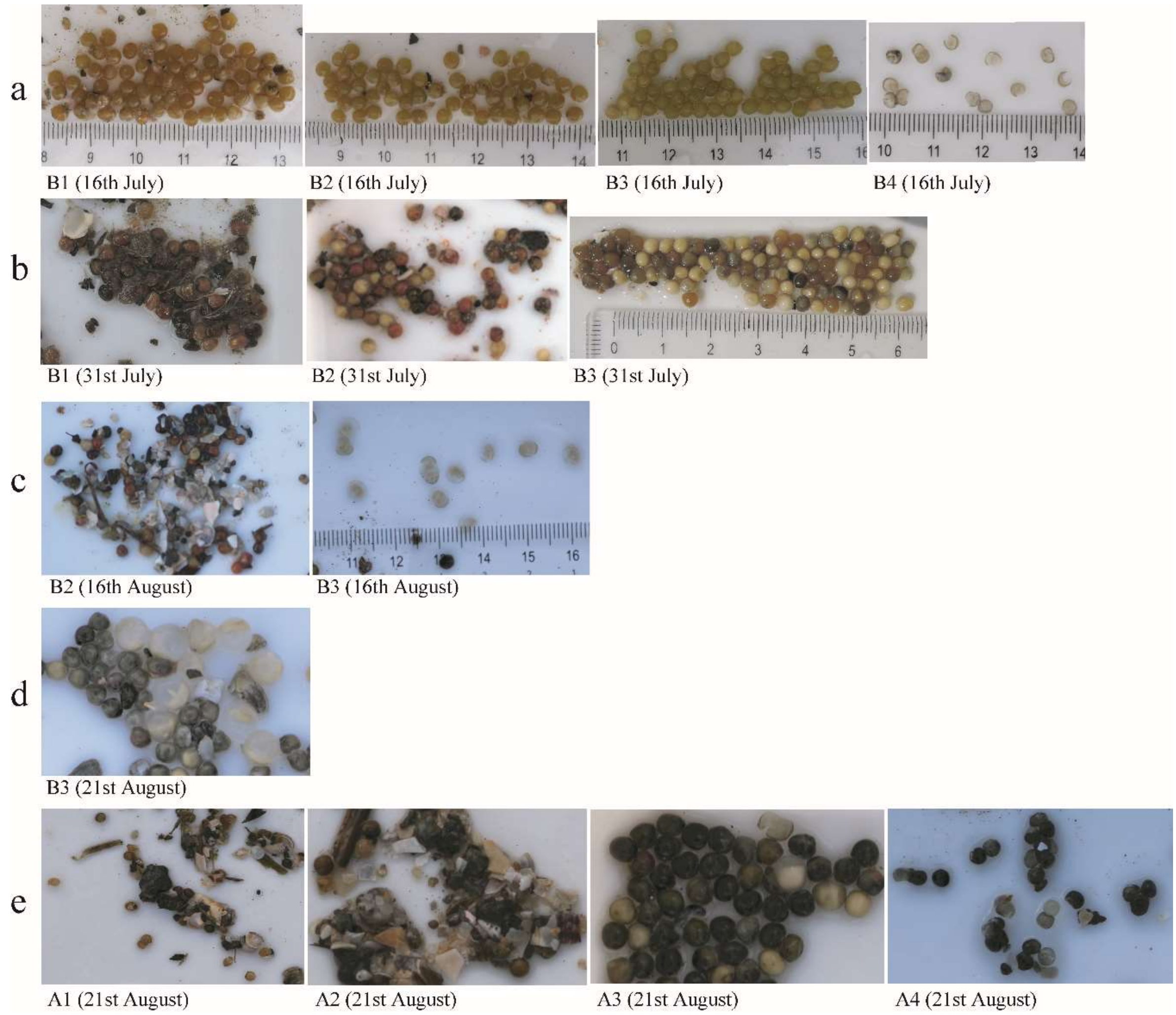
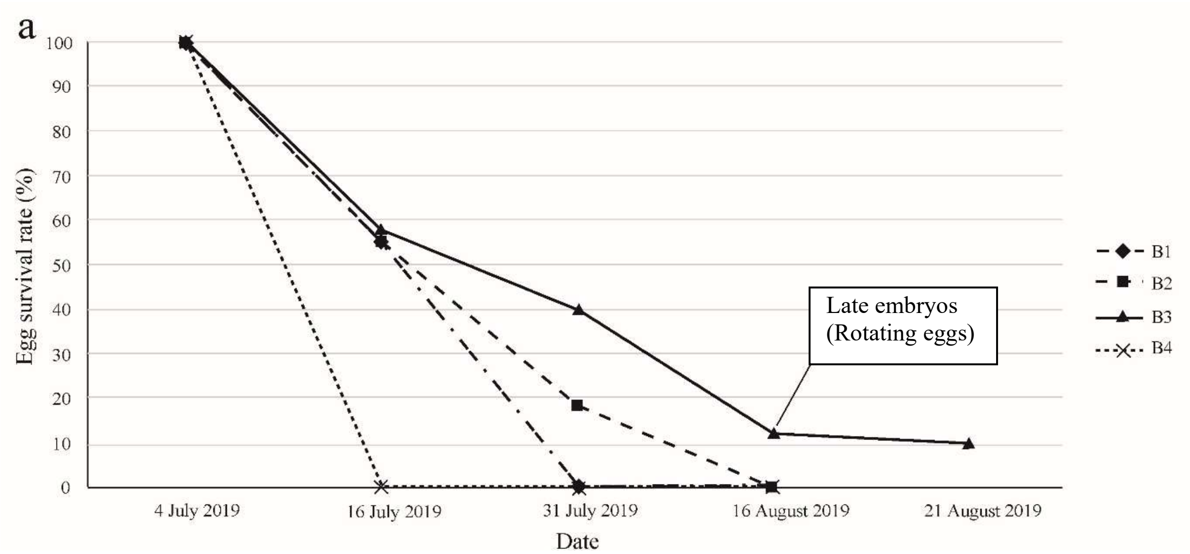


Figure 4.3 Egg survival status over the study period. a) All eggs were dead in the low intertidal zone at transect B on July 16, 2019. b) On July 31, 2019, no live eggs were found in the high intertidal zone at transect B, and reddish eggs were found in the high and mid intertidal zones. c) On August 16, 2019, all eggs died in the upper-mid intertidal zones, but the eggs in the lower-mid intertidal zones became "rotating eggs" at transect B. d) At transect B, in which the eggs were inspected regularly, 10% survived as "rotating eggs" on August 21, 2019. e) On August 21, 2019, "rotating eggs" were found only in the mid intertidal zone at transect A, which was not inspected regularly. The survival rate in the mid intertidal zone at transect A was 1%.



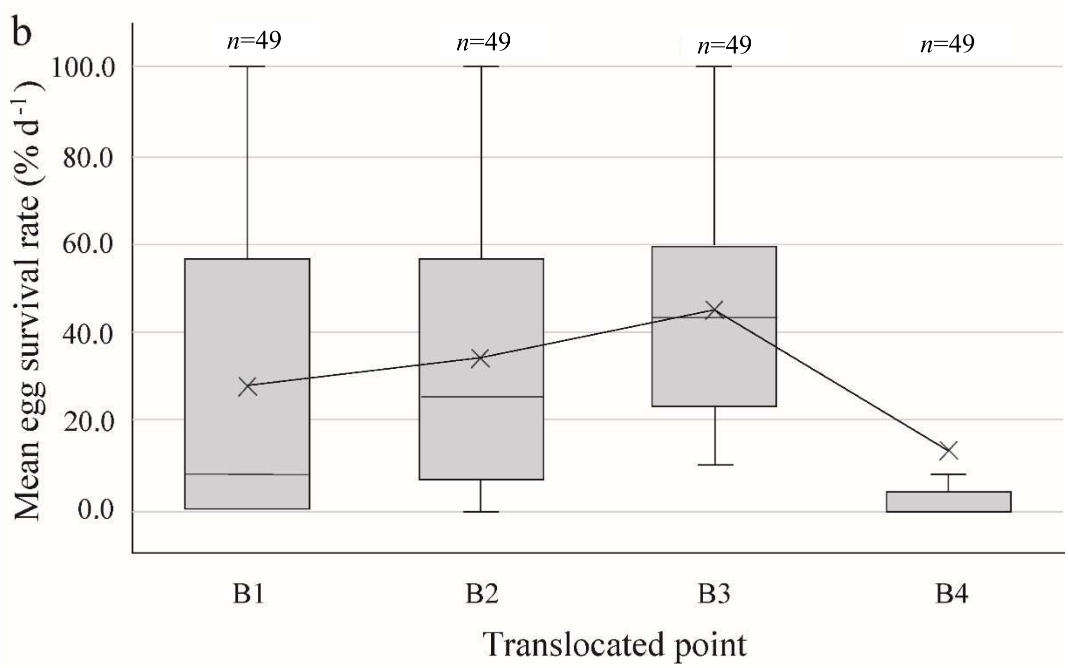


Figure 4.4 a) Changes in the survival rate of translocated eggs at transect B over the study period. b) Comparison of mean egg survival rates per day at transect B over the study period.

4.3.2 Physical Parameters

Air exposure time, water content, salinity, nest temperature, and sediment were investigated to understand the relationship between egg viability and the physical characteristics of the translocation site. The results are described below. The comparison of physical parameters in the study of this chapter and the previous studies is shown in Table 4.1.

The high intertidal zone (B1) was exposed to the air for almost 24 hours, whereas the low intertidal zone (B4) was submerged for almost 24 hours. The mean water content was low at higher elevations, and it was high at lower elevations. The mean salinity of seawater near the translocation site was $\bar{x}$=28.5±3.7 (minimum 2.8, maximum 31.5, n=2001). The mean nest temperature was the highest in the high intertidal zone and the lowest in the low intertidal zone. The sediment characteristics at the translocation site are shown in Table 4.1. For the grain size composition, fine sand accounted for about 90% at B1 and B2. At B3 and B4, medium sand and fine sand each accounted for about 50%. Thus, all translocation points were composed of sand. The median diameters were 0.17 mm for B1 and B2, and 0.26 mm for B3 and B4, respectively. The grain size sorting was from φ = 0.45 (B1) to 0.76 (B4). The silt-clay content was from 0.09% (B1) to 0.31% (B4).

Table 4.1 Comparison of the physical parameters of *T. tridentatus* spawning grounds in the study of this chapter and previous studies.

Studies		Inter-tidal zone	Air exposure time (Hour)	Water content (%)	Salinity (PSU) †	Nest Temperature (°C)	Sediment characteristics				Area
							Grain size classification *3	Median diameter (mm)	Grain size sorting (φ)	Silt-clay content (%)	
The study of this chapter	B1	High	23.3 ± 0.2	8.8 ± 1.3	28.5 ± 3.7	29.5 ± 3.0	FS	0.17	0.45	0.09	Tsuyazaki, Japan
	B2	Mid	18.6 ± 0.3	14.0 ± 2.1	28.5 ± 3.7	28.6 ± 2.8	FS	0.17	0.50	0.06	
	B3	Mid	11.9 ± 0.1	21.7 ± 1.8	28.5 ± 3.7	27.4 ± 2.5	FS / MS	0.26	0.70	0.11	
	B4	Low	3.3 ± 0.3	29.0 ± 0.6	28.5 ± 3.7	27.1 ± 2.5	FS / MS	0.26	0.76	0.31	
Botton et al., 1996					33.0			0.54 – 0.76	1.06 – 1.36		Imari, Japan
Botton et al., 1996								0.33	1.15		Morie, Japan
Sekiguchi, 1999					18.0 - 33.0	22.0 - 31.0					Imari, Japan
Maeda et al., 2000					17.3 - 26.6	25.7 – 27.6		0.40 - 1.00			Morie, Japan
Seino et al., 2000							CS	0.42 - 0.97		< 3%	Morie, Japan
Seino et al., 2001							CS	0.52 - 1.10		1.0 - 2.0	Nakatsu, Japan
Chen et al., 2004				9.8 - 13.7			MS - CS	0.40 - 1.80			Kinmen, Taiwan
Ohtsubo et al., 2005			12.0 - 17.0	5.0 - 25.0	24.0 - 29.0	26.2	FS - CS			Negligible	Imazu, Japan
Hsieh & Chen, 2009				3.7 - 9.3			CS	1.10			Miaoli, Taiwan
Wada et al., 2010							FS - G	0.20 - 5.00	0.37 - 1.98	0.0 - 6.1	Tsuyazaki, Japan
Iida et al., 2017								0.74 - 4.00			Sone, Japan

Notes: B1 to B4 are the egg translocation points in the study of this chapter. †Seawater salinity near the survey site. G, for granule; CS, coarse sand; MS, medium sand; FS, fine sand. The blank spaces indicate no data available.

4.3.3 Relationship between Egg Survival Rate and Physical Parameters

The results of analyses of the mean egg survival rate per day and the physical parameters using generalised linear models (GLMs) are shown in Table 4.2. The following parameters significantly influenced the mean survival rate of the eggs: the elevation, the mean air exposure time, the mean water content, the mean nest temperatures, grain size-sorting and silt-clay content, respectively (Table 4.2). Of these factors, the nest temperatures, grain size-sorting and silt-clay content were excluded from further analysis as these parameters were within the known range of *T. tridentatus* spawning grounds (Table 4.1). Thus, elevation, air exposure time, and water content were selected to predict the egg survival rate.

The change in the predicted values of the mean egg survival rate per day with elevation, air exposure time, and water content are shown in Figure 4.5. Of all these three physical factors, the estimated egg survival rate per day was the highest in the mid intertidal zone (B2 and B3) and the lowest in the low intertidal zone (B4) (Figure 4.5).

Table 4.2 GML analysis results for the relationship between mean egg survival per day and physical parameters.

Explanatory variable	Estimate	*z* value	Probability (>|*z*|)
Elevation	4.506	4.511	6.44e-06 ***
squared	-3.016	-4.239	2.24e-05 ***
intercept	-1.994	-6.311	2.78e-10 ***
Air exposure time	0.342	4.608	4.06e-06 ***
squared	-0.011	-4.339	1.43e-05 ***
intercept	-2.818	-6.046	1.49e-09 ***
Water content	0.402	4.024	5.73e-05 ***
squared	-0.011	-4.313	1.61e-05 ***
intercept	-3.745	-4.467	7.94e-06 ***
Salinity	NA	NA	NA
squared	NA	NA	NA
intercept	-0.847	-7.766	8.13e-15 ***
Nest temperature	31.294	2.852	0.00434**
squared	-0.552	-2.845	0.000444**
intercept	-443.880	-2.864	0.00418**
Median diameter	-1.059	-0.436	0.663
squared	NA	NA	NA
intercept	-0.620	-1.168	0.243
Grain size-sorting	113.323	3.806	0.000141 ***
squared	-95.564	-3.839	0.000123 ***
intercept	-33.090	-3.861	0.000113 ***
Silt-clay content	26.756	2.494	0.01265 *
squared	-78.311	-2.996	0.00273 **
intercept	-2.391	-3.097	0.00195 **

Notes: NA, not available. ****P*<0.001, ***P*<0.01, **P*<0.05.

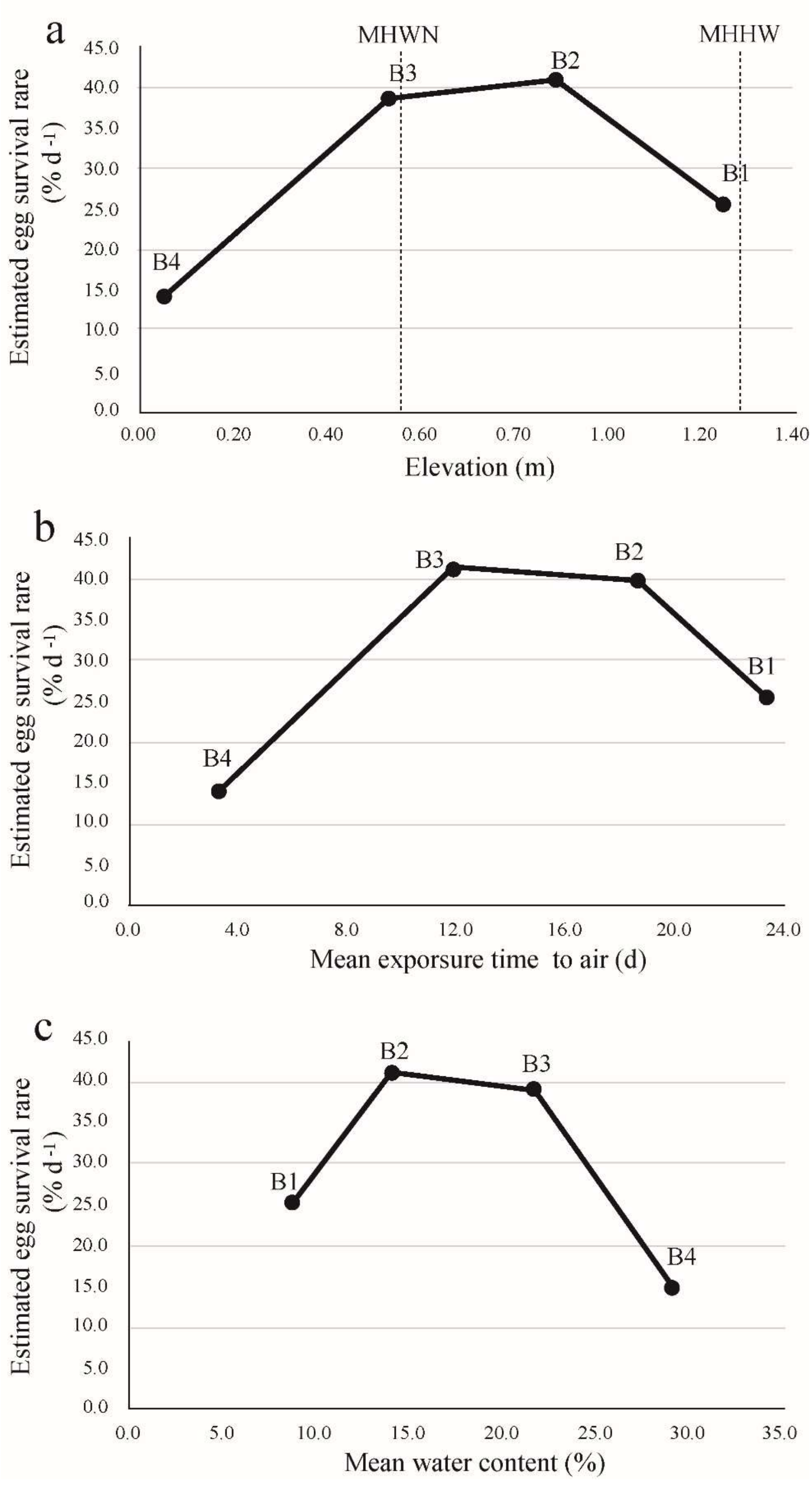


Figure 4.5 Change in estimated mean egg survival rate per day with significant physical parameters at the translocation site. a) Change in mean egg survival rate per day with different elevations; b) Change in mean egg survival rate per day with different mean air exposure time per day; c) Change in mean egg survival rate per day with different mean water contents.

4.4 Discussion

4.4.1 Egg Survival Rate

The eggs translocated at the mid intertidal zone became "rotating eggs" (stage 21) after 44 days (August 16, 2019) at $\bar{x}$=27.40±2.52 °C and salinity of $\bar{x}$=28.5±3.6 (Table 4.1 and Figure 4.4a). The results aligned with a laboratory experiment conducted by Sekiguchi (1999), where captive *T. tridentatus* eggs reached stage 21 in 43 days under conditions of 30 °C temperature and salinity ranging from 20 to 35. Egg development is believed to follow a similar rate in the field when environmental factors such as temperature and salinity are comparable (Maeda et al., 2000). Furthermore, parallel to the translocated eggs, the "wild eggs" naturally deposited in the mid intertidal zone of the same beach exhibited a similar developmental pattern. These "wild eggs," laid on July 2, 2019, progressed to the rotating egg stage by August 16, 2019. Consequently, it can be said that the translocation experiment represents the average developmental rate observed in *T. tridentatus* eggs.

The translocated eggs had the highest survival rate in the mid intertidal zone. Survival of eggs decreased at higher and lower elevations, especially "inferior" in the low intertidal zone (Figures 4.3 and 4.4). Ideally, more replications would have been desirable for the experiment. However, due to the rapid decline of *T. tridentatus* and the limited availability of spawning pairs at the study site (Itaya et al., 2019b; Itaya et al., 2022), it was challenging to obtain sufficient eggs for the experiment. Given the small number of replications available, caution should be exercised when interpreting the results of the egg survival rate. It is necessary to conduct a follow-up experiment with a larger number of replications involving translocated eggs to ensure the validity of the current findings. Nevertheless, it can be inferred that significant variations in egg survival rates existed across different elevation points (Figure 4.4). Furthermore, the application of GLM modelling revealed that the elevation gradient from high to low tidal zones substantially influenced the egg survival rate (Figure 4.5a), indicating its importance for further attention. The egg survival rate was the highest in the mid intertidal zone (B2 and B3). Of these eggs, 10% of B3 eggs finally developed to "rotating eggs" (Figure 4.4a). There is almost no field-based research on the survival rate of *T. tridentatus* eggs in the wild. In Taiwan, Hsieh & Chen (2009) found that the hatching rate ranged from 0 to 88.5%, with an average of 33.9%, and hatching rates declined in places with small tidal amplitude (i.e., the lower intertidal zone). In the experiment of Hsieh & Chen (2009), there were three spawning areas, A, B, and C, on the same beach. Still, the above survival rate was calculated for only four nests in area A, where hatching was confirmed (the reason for the exclusion of areas B and C is unknown). However, if areas B and C, where hatching was not confirmed, were included, the total number of nests would be twelve. Then, the average survival rate of 33.9% found by Hsieh & Chen (2009) could be divided by twelve nests instead of four nests, and then the survival rate would be about 11%. The egg survival rate in the mid intertidal zone (B3) of the study of this chapter was 10% (Figure 4.4a), which is almost the same as their conversion value. In addition, when considering all of the translocated eggs in transect B, the number of surviving eggs is 10 out of 400, and the survival rate is 2.5%. In summary, the range of egg survival rate in the study of this chapter is from 2.5% (minimum) to 10% (maximum), which is not essentially different from the case reported by Hsieh & Chen (2009). Therefore, it is assumed that the survival rate of *T. tridentatus* eggs obtained in this chapter may reflect the survival rate in the wild.

On the other hand, some reddish eggs showing bacterial infection in the translocated eggs were found (Figure 4.3). Sekiguchi (1999) removed infected eggs every time in his laboratory experiment. However, removing the infected eggs in the hydroponic pots was not performed to reduce human influence as much as possible. It is more likely that infection can spread quickly within the closed environment of the hydroponic pots. Thus, this may have led to a decrease in the survival rate of the translocated eggs.

Several studies have demonstrated the spawning location of horseshoe crabs within the intertidal zone. In Imari, Japan, 97% of *T. tridentatus* spawning was found between the MHHW and the MHWN, but most were concentrated near the mid intertidal zone (Sekiguchi, 1999). In Malaysia, more than 50% of *T. tridentatus* spawning occurred in the mid intertidal zone (Mohamad et al., 2019). In the study of the Atlantic horseshoe crab (*L. polyphemus*), egg development increased in high and mid intertidal zones, but it decreased in the low intertidal zone (Vasquez et al., 2015). Another study pointed out that *L. polyphemus* spawned in the mid intertidal zone, which maximised their egg development rate by avoiding the dry and hot environment in the high intertidal zone and the oxygen-deficient condition in the low intertidal zone (Penn & Brockman, 1994). The present findings align with the results of these previous studies, indicating that the translocated eggs exhibited the highest survival rate in the mid intertidal zone (Figures 4.4 and 4.5). Thus, despite the lack of specific research focusing on *T. tridentatus* egg development across different elevations along the high-to-low tide gradient, it can be concluded that *T. tridentatus* prefers the mid intertidal elevations as their favoured spawning location.

4.4.2 Physical Parameters Contributing to Egg Survival Rate

Given *T. tridentatus* prefers spawning around the mid intertidal zone, the total time the eggs are submerged is relatively short. The air exposure time of eggs at the translocated site was longer at higher elevations of the intertidal zone. Consequently, the water content was lower at higher elevations and higher at lower elevations (Table 4.1). However, due to the sandy beach's water storage capacity and the egg's water absorptive function, seawater is retained in the egg even when the beach is exposed to air (Sekiguchi, 1999). Penn & Brockman (1994) found that the water content of sand is one factor that influences egg development in horseshoe crabs. In the study of this chapter, the water content of the eggs at each translocated point was determined by the tidal movement and the degree of elevation of the intertidal zone.

In the mid intertidal zone (B2, B3), where egg survival was higher, the mean air exposure time was 11.9 to 18.6 hours per day (Table 4.1). Although very few studies quantified the air exposure time of spawning grounds, it was 12.0 to 17.0 hours per day in Imazu, Japan (Ohtsubo et al., 2005), which is almost the same as the results of the study of this chapter (Table 4.1). On the other hand, in the high intertidal zone (B1) and the low intertidal zone (B4) where egg survival was relatively poor, B1 was exposed to air for almost 24 hours and B4 was flooded almost all day (Table 4.1).

The egg survival rate was the lowest in the low intertidal zone (B4) (Figure 4.4). This trend was also evident in the GLM models, which showed that the air exposure time and water content contributed significantly to the egg survival rate (Table 4.2; Figure 4.5b and c). Ohtsubo et al. (2005) pointed out that the low intertidal zone is unsuitable for spawning due to the shortage of dissolved oxygen in the pore water in moist sand. A sandy beach takes in abundant oxygen at low tide when exposed to the air. Then, when it immerses in seawater at high tide, the oxygen in the sand dissolves into the seawater and finally is absorbed by the eggs. Therefore, *T. tridentatus* eggs develop better when the seawater is completely removed at a certain frequency than when they are constantly immersed in seawater (Sekiguchi, 1999). Thus, the low egg survival rate in the low intertidal zone can be explained by the lack of air exposure time, resulting in excessive water content (Table 4.1), which causes insufficient oxygen for the eggs.

The water content in the high intertidal zone (B1) was within the range of other studies (Table 4.1). However, in the high intertidal zone, the water content temporarily increased during high tides but remained low over the other tidal periods. Furthermore, while other translocated points were submerged in seawater at least once a day, there were three times when the high intertidal zone was not submerged for more than a week during the study period. It accounted for 62% of the total time when the high intertidal zone was not immersed in seawater. In a laboratory experiment with the coastal horseshoe crab (*T. gigas*), Faizul et al. (2013) showed that eggs in sand develop well when immersed in seawater at a frequency of one to three days. It was suggested that when the eggs are not submerged in seawater for more than a week, desiccation may cause a poor egg development rate (Faizul et al., 2013). Therefore, in the study of this chapter, it is possible that dissolved oxygen did not reach the eggs sufficiently in the high intertidal zone as the immersion time and its frequency were extremely low. Also, the desiccation of the eggs may have occurred due to the lack of water content over the study period.

In contrast, the mid intertidal zone (B2 and B3) was covered in seawater at a moderate frequency, and it was exposed to air for about 30 to 50% of each day. It, in turn, would have made a sufficient supply of oxygen for egg development possible, leading to the highest egg survival rate. Thus, the mid intertidal zone is considered the most suitable spawning zone for *T. tridentatus* with respect to air exposure frequency and water content.

4.4.3 Management Implications of the Findings

The results of the field-based study in this chapter can be applied to the restoration and conservation of the spawning ground for the critically endangered horseshoe crab, where the suitable spawning grounds are limited because of coastal development. The study of this chapter revealed that the egg survival rate in the low intertidal zone was meagre (Figures 4.4 and 4.5). Some spawning pairs of the area laid eggs in the low intertidal zone, suggesting there was probably a shortage of suitable spawning habitat (Itaya et al., 2019a). Hence, the egg survival rate of some of the spawning pairs is insufficient to sustain a viable population of horseshoe crabs in Tsuyazaki Cove. It may be necessary to translocate eggs laid in the low intertidal zone to the mid intertidal zone to increase their survival rate. However, as described below, the translocation of the eggs alone may not be sufficient for the recovery of the natural population in Tsuyazaki Cove.

The sandy spawning grounds in Tsuyazaki Cove have experienced a reduction in sand supply due to coastal development, resulting in a rapid decline in spawning pairs from 2005 to 2013, particularly after 2009, when more than 90% of the population vanished. Currently, the number of spawning pairs coming to the beach every year is only four to ten pairs (Itaya et al., 2019b; Itaya et al., 2022). Thus, the spawning grounds at Tsuyazaki Cove have degraded over time, and the natural recovery of the spawning grounds and the population cannot be expected. Therefore, protecting the remaining suitable spawning sites and restoring those degraded sites is urgent. Securing the mid intertidal zone is critical to maximising the egg survival rate in Tsuyazaki Cove by protecting what remains from it and recovering other sites for spawning. It is even possible to design and build artificial spawning sites. For these practical conservation measures, further research studies will be necessary to estimate the remaining suitable spawning grounds and those degraded ones. This is especially important for the recovery strategies of specific species.

4.5 Conclusion

The study of this chapter provided profound insights into the relationship between *T. tridentatus* egg survival and the physical characteristics of the spawning ground for the first time. The results emphasised the vital role of spawning beach elevation relative to tidal range in determining the viability of *T. tridentatus* eggs. The mid intertidal zone emerged as the most suitable habitat for egg survival, offering optimal conditions such as appropriate air exposure time, water content, and sediments. The duration of beach exposure to air and the water content in the sand, influenced by the difference in beach elevation, were found to have a significant impact on the survival of the eggs. Higher and lower intertidal zones, with excessive or insufficient air exposure time, negatively impacted egg survival. The mid intertidal zone exhibited a dynamic pattern of moderate saturation and dehydration with each tidal movement, which proved crucial for the survival of *T. tridentatus* eggs.

Consequently, protecting mid intertidal zones as suitable spawning areas holds significant importance for the future management and conservation of this globally endangered species. Furthermore, the translocation of eggs from marginal zones to more suitable spawning zones has potential as a strategy for population recovery. The chances of successful hatching and population growth may increase by relocating eggs from areas with suboptimal conditions to zones that provide more favourable environments for their development and survival. This approach may help enhance the species' overall reproductive success and resilience.

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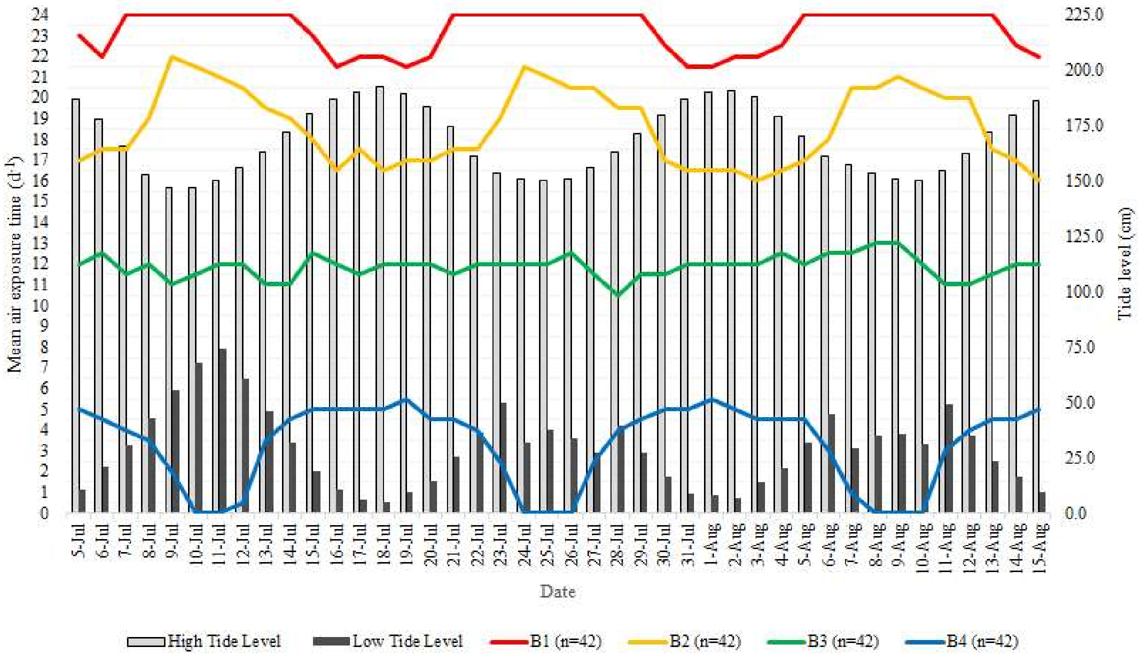
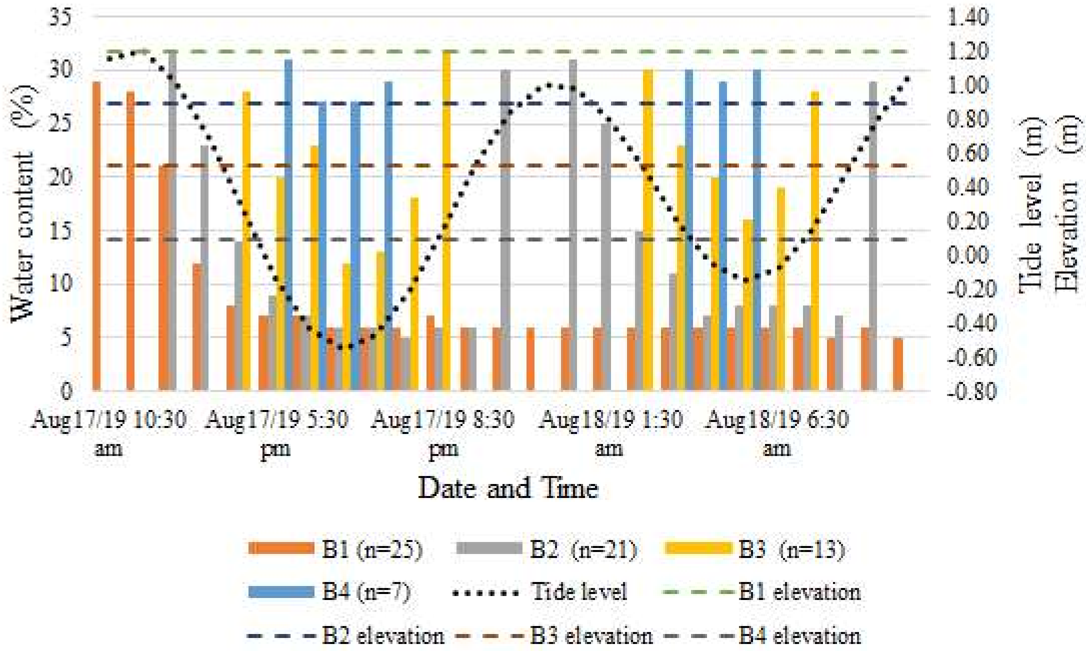
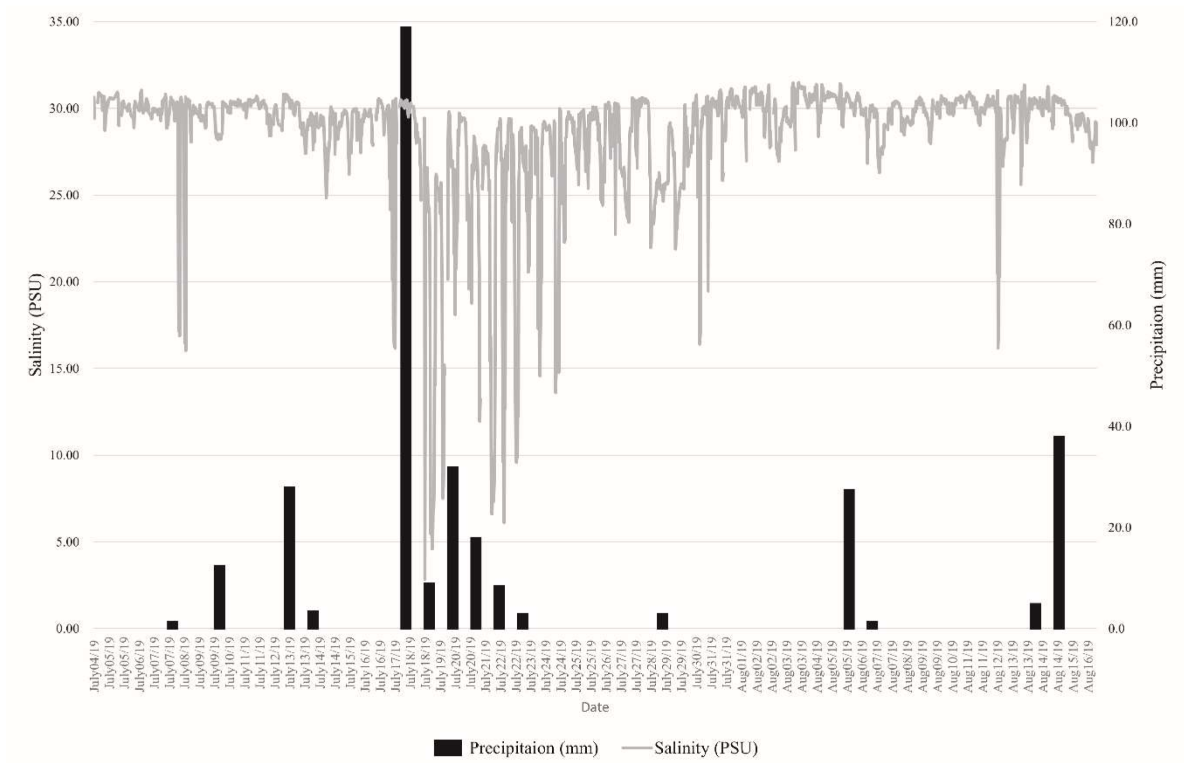
Appendices



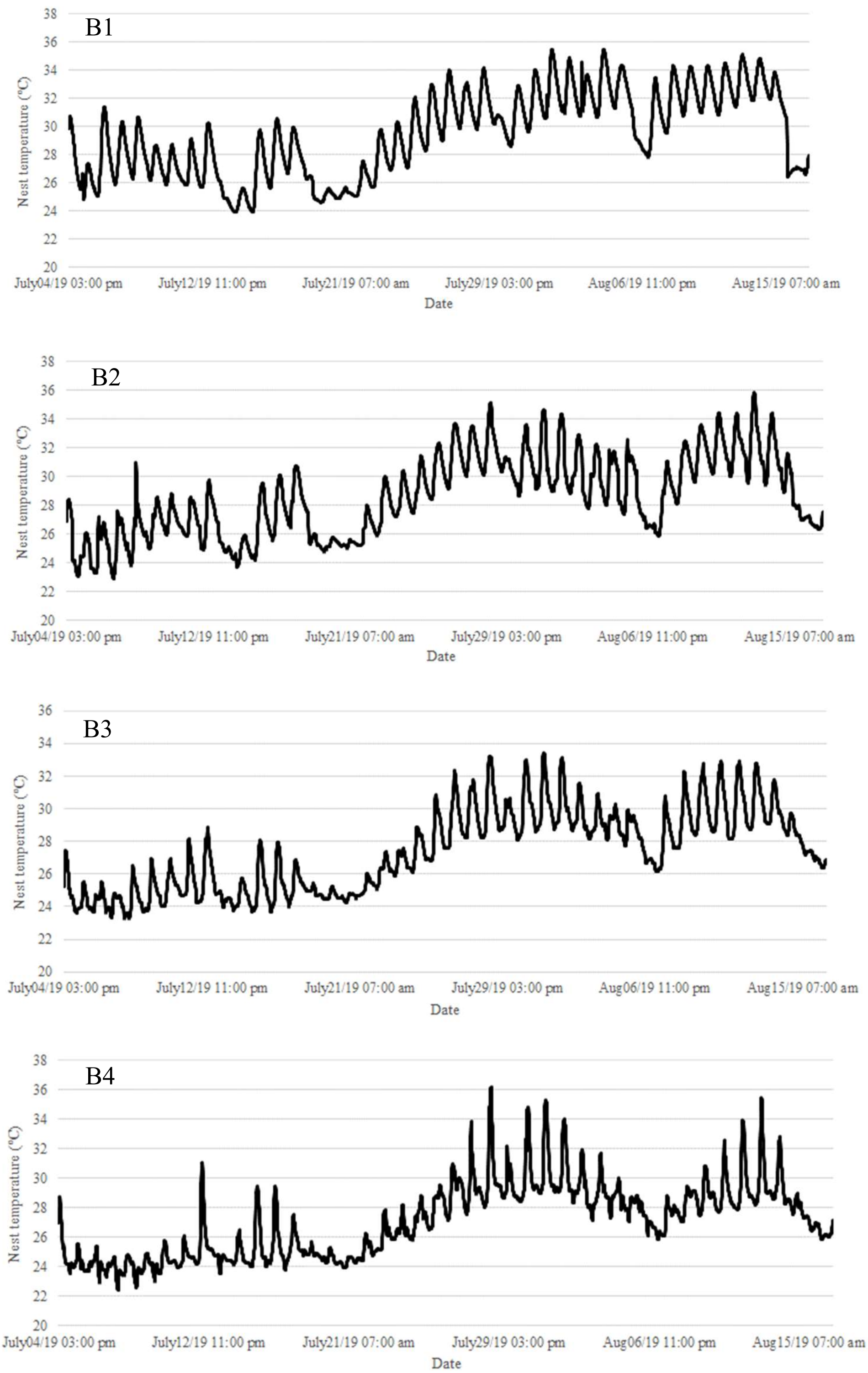
Appendix 4.I Changes in mean air exposure time per day at each translocation point (B1 - B4) with the tide movement over the study period.



Appendix 4.II Changes in water content at each translocation point (B1 - B4) with tidal movement from August 17 to 18, 2019.



Appendix 4.III Changes in salinity with precipitation at the translocation site over the study period. Precipitation data were obtained from Japan Meteorological Agency.



Appendix 4.IV Changes in nest temperature at each translocation point over the study period.

Chapter 5

Mapping Spawning Habitat Suitability for the Conservation of the Tri-spine Horseshoe Crab *Tachypleus tridentatus*

Abstract The suitable spawning habitat of *Tachypleus tridentatus* was estimated and mapped for the first time using scientific modelling in Tsuyazaki, Fukuoka, Japan. A total of 43 nests were found at five sites from 2018 to 2019. Of these 43 nests, the location information of nine nests at one of the sites was used as "Training data" for constructing models. A series of procedures was employed to determine the relationship between the biological data and their physical parameters to estimate the suitable spawning habitat. First, the contribution rates of the physical parameters to nest locations were evaluated. Second, physical parameters that showed significant contribution rates were chosen to estimate suitable spawning habitats. It was found that the beach elevation relative to the tidal range was the most significant parameter for estimating suitable spawning habitats. MHWN (Mean High Water Neap) to MHHW (Mean Higher High Water) was estimated as the most suitable spawning zone, and 74 % of the nests were fitted in an area assessed as suitable. The result was consistent with previous studies. The area of suitable spawning habitat was estimated to be only 476 m^2 out of 1943 m^2, comprising 24 % of the sandy beach in the study site. Therefore, it was concluded that the conservation and restoration of spawning grounds is a high-priority and urgent issue at the site. It is proposed that scientific modelling of nest/egg occurrence and beach elevation relative to the tidal range can effectively determine suitable spawning habitats. Thus, it is possible to quickly establish the habitat most suitable for spawning in coastal environments and plan conservation strategies for this globally endangered species.

Keywords: coastal development, globally endangered species, horseshoe crab conservation, Maxent analysis, spawning habitat suitability model

5.1 Introduction

Due to the susceptibility of *Tachypleus tridentatus* spawning habitats (i.e., sandy beaches in calm semi-enclosed bays) to human development, the loss of these environments has been one of the most significant causes of the reduction of the *T. tridentatus* populations (Sekiguchi, 1989; Sekiguchi, 1999). Thus, strategic research and practical measures for conserving this species, in particular, the conservation and restoration of the spawning habitats, are the most critical issues for the survival of this species (Hsieh & Chen, 2009; Wada et al., 2010; Itaya et al., 2019b; Itaya et al., 2022a).

Many studies have investigated the environmental conditions necessary for the successful spawning of *T. tridentatus*. For example, beach elevation relative to the tidal range, slope, grain composition, sediment moisture, exposure time, and dissolved oxygen have all been suggested to be environmental factors that determine the nesting location (e.g., Seino et al., 1998; Ohtsubo et al., 2005; Wada et al., 2010; Hsieh & Chen, 2015; Mohamad et al., 2019). It is also known from observations in captivity and in the field that appropriate salinity and temperature conditions are required for egg development and larval hatching (Sugita et al., 1985; Sekiguchi, 1999; Maeda et al., 2000; Chen et al., 2004). However, these studies report only on the environmental conditions of the spawning site (or physical factors related to egg development in the case of captivity). A thorough analysis of the relationship between biological data (nest/egg occurrence) and related physical parameters, as well as the construction of a habitat suitability model (HSM) using these data, has not been performed. In addition to supporting conservation planning for endangered species, HSM facilitates habitat mapping, which is essential for planning effective conservation and establishing nature reserves (Gogol-Prokurat, 2011; Koljonen et al., 2013; Onikura, 2015). For horseshoe crabs facing extinction at the local level, as in the study of this chapter, scientific modelling, estimating suitable habitats using the analytical data set and generating habitat suitability maps for spawning are seen as vital for practical conservation measures. First, they illustrate the precise areas needing protection. Second, they become an important means of communication whereby decision-makers can easily visualise suitable habitats for spawning.

T. tridentatus spawns in the intertidal zone between the mean higher high water (MHHW) and the mean high water neaps (MHWN) (Sekiguchi, 1999) (Figure 5.1). However, *T. tridentatus* that have lost suitable spawning sites due to coastal development may lay eggs lower than the MHWN (Sekiguchi, 1999). Coastal development has decreased the number of breeding pairs in Tsuyazaki by removing a large area of sandy beach suitable for spawning (Itaya et al., 2019b; Itaya et al., 2022b). Nesting lower than the MHWN was also found in the area (Itaya et al., 2019a). Therefore, in this chapter, known spawning sites in Tsuyazaki Cove were evaluated in terms of their habitat suitability using scientific models. In addition, the estimated suitable spawning habitats remaining in the area were mapped. Based on these results, recommendations for ways to conserve and restore the remaining spawning grounds are discussed.

5.2 Methods and Materials

5.2.1 Study Site

The study was conducted at Tsuyazaki Cove in Fukuoka (Figure 5.2). Numerous studies on *T. tridentatus* have been conducted in the cove, including the juvenile population (Wada et al., 2008), the suitable juvenile habitat (Koyama et al., 2020), the adult habitat and movement patterns (Wada et al., 2016), the spawning population (Wada et al. 2010; Itaya et al. 2019b; Itaya et al., 2022b), the spawning environment (Itaya et al. 2019a; Itaya et al., 2022a). According to Wada et al. (2010), Sites 1 - 4 are essential for the local population as about 70 % of *T. tridentatus* spawning is concentrated on these small beaches (Figure 5.2).

In Tsuyazaki, 80 % (from 26,303 m^2 in 1948 to 5,376 m^2 in 2010) of sandy beaches disappeared due to coastal development. In particular, the artificial closure of the bay mouth by constructing a yacht harbour and associated embankment, completed in 2005 - 2006, left only about 60 m of space between the cove and the open sea. As a result, the supply of sand being transported from the open sea to the spawning beaches has ceased, leading to a decrease of more than 90% of spawning *T. tridentatus* in the area (Itaya et al., 2019b; Itaya et al., 2022b).

5.2.2 Digital Topographical Mapping and Tidal Level Measurement

The beach measurement was conducted during the low tides and completed with two people for three days (actual working hours less than 18 hours). The beach elevation and latitude/longitude data were obtained using the Real-Time Kinematic-Global Navigation Satellite System (RTK-GNSS: Trimble R4 GNSS, Nikon-Trimble Co., Ltd.) at Sites 1 - 4 during the spawning season. The beach elevation based on T.P. (Tokyo Peil: the average sea level in Tokyo Bay) as 0 m, the standard for expressing elevations in Japan, was used in this chapter. Beach elevations are expressed relative to T.P., equivalent to tidal level (specific heights of the shoreline affected by tides) throughout this chapter. These data were then imported into ArcGIS (ArcMap version 10.8, ESRI Japan Co., Ltd.) to create three-dimensional digital maps for each site. The undulation maps of each site were created based on the three-dimensional maps using the ArcGIS tool

"Slope", in which each cell (mesh size 0.2 m) of the raster surface elevation was employed to calculate the gradients of the nesting sites. Aerial photographs of each site were taken using a drone (Spark, Dji Co., Ltd.), which were georeferenced and then overlaid with the digital maps in ArcGIS.

A portable water depth meter (COMPACT-TD ATD-HR, JFE Advantech Co., Ltd.) was used to obtain the fluctuation of tidal levels at the study site. Then, the tidal ranges based on the definition of the Japan Coast Guard (2022) were calculated. For example, MHHW (Mean Higher High Water) was calculated as the sum of the sea levels of the highest tides during the study period divided by the number of times the highest tides were observed. The tidal range is shown in Figure 5.1.

5.2.3 Nest Distribution Survey

Sites 1 - 4 were surveyed to determine nest locations (i.e., biological distribution data) (Figure 5.2). The surveys were conducted on high tides of flood tides from July to August in 2018 and 2019 (26 days in total), which is the primary spawning season of *T. tridentatus* in Japan. Each survey was carried out from two hours before to one hour after the highest tide, based on the times of high tide twice in the morning and at night (six hours per day). Nest locations were marked by plastic stakes placed next to the breeding pairs remaining unmoved when the female *T. tridentatus* started laying eggs (The measurement of the nest locations is explained in the next section). In total, 43 nests were found in the study area (*n*=7 at Site 1, *n*=9 at Site 2a, *n*=5 at Site 2b, *n*=9 at Site 3, *n*=13 at Site 4).

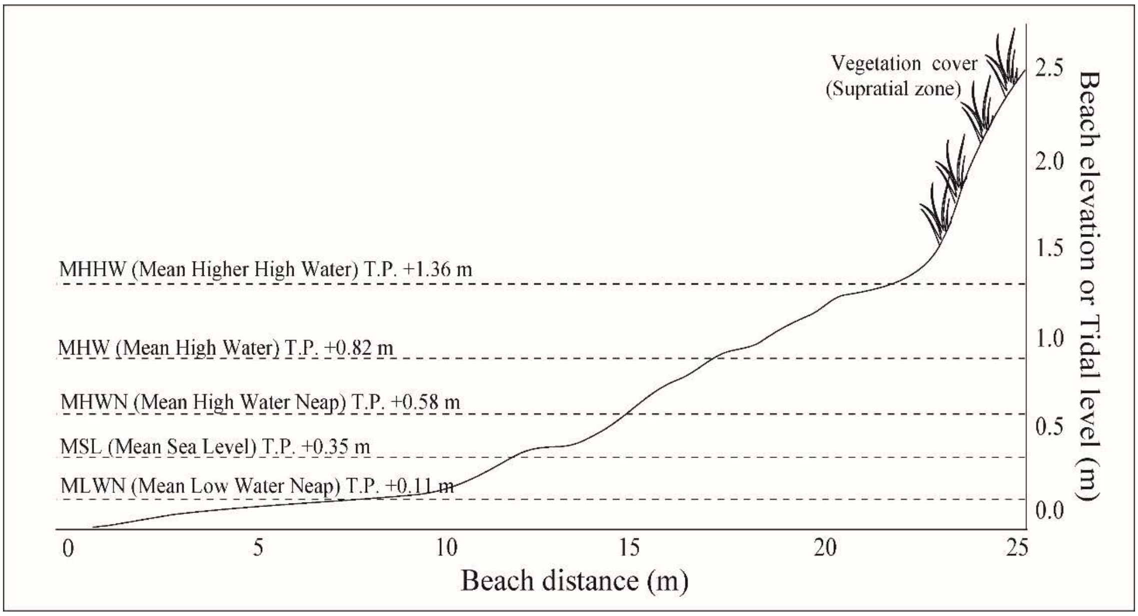


Figure 5.1 Tidal ranges and tidal zones at the study site. The elevation (or tidal level) is based on T.P. (Tokyo Peil: the average sea level in Tokyo Bay) as 0 m. The abbreviations follow the definition of the Japan Coast Guard (2022). The 0 m of the beach distance was the bottom of the beach where the slope and sediment composition changed abruptly (i.e., where the sandy beach and the sandy mudflat meet). The diagram illustrates the cross-section of Site 2a.

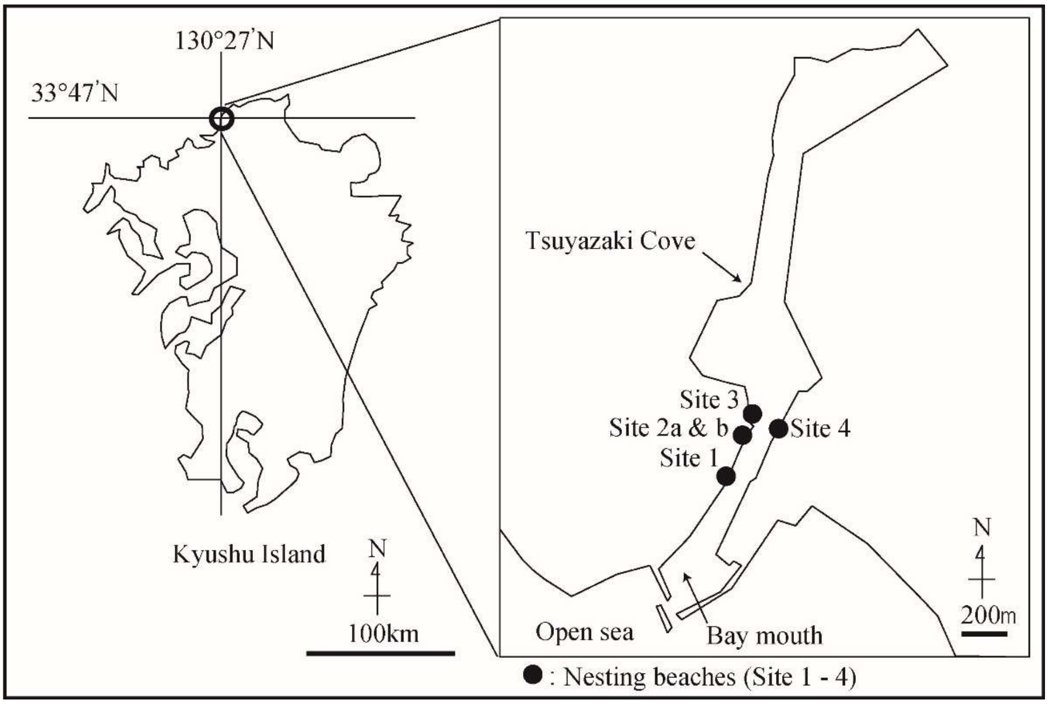


Figure 5.2 Location of the study site in Tsuyazaki, Fukuoka, Japan. Dots indicate nesting beaches.

5.2.4 Environmental Data Collection

T. tridentatus nests' sediment moisture, exposure time and dissolved oxygen in pore water in the target area are known to depend on the relative relationship between beach elevation and tidal range (Itaya et al., 2022a). Therefore, beach elevation was considered the most important parameter that indicates a suitable spawning habitat. Since it is known that the range of salinity (28.5±3.7 PSU) and nest temperature (27.1 - 29.5 ℃) in the spawning grounds of the target area is not a factor that inhibits egg development (Itaya et al., 2022a), these environmental factors were excluded. Beach slopes can be a cue for finding spawning sites (Botton & Loveland, 1987), and differences in beach slopes could stimulate horseshoe crab spawning (Nelson et al., 2019). The location of *T. tridentatus* nests ranges at slopes of 3.29 - 8.26° (Seino et al., 1998; Itaya et al., 2019a), indicating that they prefer gradual steepness. Although *T. tridentatus* lays eggs on sandy beaches encompassing various grain sizes, from fine to gravel, for spawning in Japan (Iida et al., 2017), nest/egg occurrence can be affected by characteristics of spawning ground sediments as demonstrated by many other horseshoe crab studies (e.g., Penn & Brockmann, 1994; Nelson et al., 2016; Botton et al., 2018). Thus, the beach elevation relative to the tidal range, beach slope, and sedimentary parameters (median diameter, grain size sorting, silt-clay content, skewness, and percentage of gravel) were selected as environmental parameters for modelling.

The elevation and latitude/longitude of each nest (the location of the plastic stakes) were measured using the RTK-GNSS during the low tides. The slopes of each nest location were extracted from the undulation maps in ArcGIS. About 100 g of the sediment 15 cm below the ground surface, the depth at which *T. tridentatus* lay eggs in the wild (Maeda et al., 2000; Chen et al., 2004), was collected at each nest with a hand shovel for grain size analysis. Further details on the grain size analysis are described below.

To compare nesting (occurrence) and non-nesting (non-occurrence) environments, non-nesting data of these three variables were also collected. Non-nesting environmental data were sampled along the MLWN line at five to ten-meter intervals within each site (n=8 at Site 1, n=5 at Site 2a, n=3 at Site 2b, n=5 at Site 3, n=6 at Site 4). Non-nesting data sampling along the MHHW line was conducted only at Site 2a (n=4), and it was not carried out for other sites where bulkheads were located below the MHHW line (i.e., no MHHW line existed). In total, 31 samples were collected as non-nesting data for each variable.

The grain size analysis for the sediments of both nesting and non-nesting sites was determined using seven gauges of sieves with a mesh size of 0.064, 0.125, 0.25, 0.5, 1.00, 2.00 and 4.00 mm. Dried sediment samples were passed through each sieve, and the grain size composition was determined from the weight of the particles remaining on each sieve and the ratio to the total weight. In addition, the median diameter, grain size-sorting, silt-clay content, skewness, and percentage of gravel were calculated for the sediment at each nesting and non-nesting point. The median diameter (*Md*) represents the average grain size of sediments, and the "median" in the grain size distribution is used for the value. The grain size-sorting (*So*) expresses the uniformity of particles. So > 1.0 indicates poorly selected sediment, and So < 0.5 means well-sorted sediment. Skewness (*Sk*) represents the asymmetry near the highest point of the grain size distribution. A perfectly symmetric grain size distribution has a skewness of 0.00. A positive strain represents an increase in small grains, and a negative strain a shift toward larger grains (Folk & Ward, 1957).

The calculations of median diameter, grain size-sorting, and skewness were based on the formulae below as suggested by Folk & Ward (1957). The silt-clay content and the percentage of gravel were calculated from the ratio of silt-clay and gravel to the total weight, respectively. Since the grain size distribution tends to be a logarithmic normal distribution, their grain sizes were classified using the phi scale (φ) in the first place. The phi scale represents a negative exponential function with 2mm as the reference. When the grain size d is expressed in millimeters, φ is calculated by the following formula:

$$\phi = -\log_2 d, \quad (5.1)$$

where d is the grain diameter in millimeter. Then, the median diameters were calculated as follows:

$$Md\phi = \phi_{50} = -\log_2 d_{50}, \quad (5.2)$$

where $Md\varphi$ and d_{50} are the grain sizes corresponding to the cumulative frequency of 50% in the grain size distribution, respectively. For the grain size-sorting (*So*) calculation, the following formula was used:

$$So = (\phi_{84} / 4) + (\phi_{95} - \phi_5)/6.6, \quad (5.3)$$

where φ_5, φ_{84} and φ_{95} are the grain sizes corresponding to the cumulative frequency of 5%, 84% and 95% in the grain size distribution, respectively. For the skewness (*Sk*) calculation, the below formula was used:

$$Sk = (\phi_{16} + \phi_{84} - 2\phi_{50})/2(\phi_{84} - \phi_{16}) + (\phi_5 + \phi_{95} - 2\phi_{50})/2\phi_{95} - \phi_5), \quad (5.4)$$

where φ_5, φ_{16}, φ_{50}, φ_{84} and φ_{95} are the grain sizes corresponding to the cumulative frequency of 5%, 16%, 50%, 84% and 95% in the grain size distribution, respectively.

5.2.5 Data Analysis and the Generation of Suitable Spawning Habitat

Two procedures in the data analysis were used. First, each environmental parameter was evaluated using generalised linear models (GLMs) using R version 3.4.4. Second, for those environmental parameters readily mapped that contributed significantly to nest/egg occurrence ($p<0.05$), maximum entropy modelling (Maxent) analysis of the geographical distribution of species was used to map suitable spawning habitats.

In the GLMs, the beach elevation, slope, and sedimentary parameters of both "nesting" and "non-nesting" data were utilised as dependent variables, and the nest/egg occurrence as a predictive variable. The square value of each physical parameter was also used as an explanatory variable to express binominal distribution (ordinary least square method).

Maxent is a versatile machine learning method with a simple and precise mathematical formulation, and it is suitable for species distribution modelling in many aspects (Phillips et al., 2006). For example, it can generate robust models even with small sample sizes (Phillips & Dudík, 2008). Maxent estimates species distribution based on the known distribution of the species (called "presence" or "occurrence" data) and environmental information such as topography and vegetation (Phillips & Dudík, 2008). The model employs an algorithm called the Maximum Entropy. Maxent randomly generates points called the background environment instead of "absence" or "non-occurrence" data in the target area. The suitable distribution of the target species is then determined from the relationship between these random points and the probability density of the environmental variables at the actual distribution (Phillips & Dudík, 2008). Maxent estimates the probability distribution $q\lambda$ defined by the following equation (Phillips et al., 2006):

$$q\lambda(x) = exp(\sum_{i=1}^{n} \lambda_i f_i(x))/Z_\lambda , \qquad (5.5)$$

where x is the target area, $fi(x)$ is the environmental variable value at point x, λi is the coefficient, and $Z\lambda$ is the constant to make the total value of $q\lambda$ at all points equal one.

Maxent software version 3.4.1 (Phillips et al., 2022) was used to estimate the spawning habitats and map suitable spawning areas for the horseshoe crab. Two types of data were used in the Maxent analysis. One was the occurrence data (i.e., the location of the horseshoe crab nests), and the other was the environmental data (i.e., the variables with high contribution rates in the GLMs). In the Maxent analysis, raster files (data consisting of pixels arranged in a grid of rows and columns) are needed for the environmental data (Phillips et al., 2006). For beach elevation and slope, it was possible to create topographical maps in raster file format from measuring points using ArcGIS (more than 1,000 points of elevation and latitude/longitude data were used for building the topographical maps in this chapter). However, such large numbers of sampling could be time and labour-consuming and, therefore, impractical in beach sediment that involves laboratory analysis and field sampling. Moreover, expressing beach sediment distributed randomly and continuously on a topographical map was not practical. Thus, variables associated with sediment were excluded from the Maxent analysis.

Pearson's correlation analysis was also applied to determine whether the explanatory variables were independent. When the independence of the explanatory variables was confirmed (correlation coefficient <0.7), then Maxent analysis on a combination of the variables as well as each variable was performed, respectively. The AUCs (the Area Under the Receiver Operating Characteristics Curve), the sensitivity (actual nesting fitness rate in the areas estimated to be suitable), and the cut-off (threshold) were calculated to evaluate the accuracy of the Maxent models. AUC values range from 0 to 1, with higher values indicating the model is more or less accurate. AUC values of more than 0.7 are considered valid, and the model is highly accurate with more than 0.9 AUC values (Swets, 1988).

It was determined that models with >0.7 AUC values were reliable in this chapter. The area with the predicted value above this cut-off was considered suitable for nesting. Then, the cut-off value was converted into the actual value of the environmental parameters for the sake of clarity. Site 2a is the only site in the study area where an elevation zone higher than MHHW remains (i.e., it is the only site where the original spawning beach shape is maintained). In addition, according to previous surveys, it supported the highest number of breeding *T. tridentatus* (Wada et al., 2010; Itaya et al., 2022b). Therefore, a model at Site 2a was first built as "Training data" in the Maxent analysis. Then, the model with the highest sensitivity and AUC was selected to extrapolate into Sites 2b, 3 and 4 as "Test data". Thus, the robustness of the model was evaluated. Finally, using the model judged to be the most robust by Maxent, the suitable spawning habitat was mapped, and the remaining suitable area was calculated.

5.3 Results

5.3.1 Beach Elevations, Slopes and Grain Composition of Nests

The beach elevations of the nests ranged from T.P. +0.12 m at Site 2b to T.P. +0.74 m at Site 2a (Table 5.1). The nests were located on beach slopes from 4.63° at Site 2b to 8.34° at Site 1 (Table 5.1). All the nests were dug on beaches composed of sand with median diameters ranging from 0.19 mm at Site 1 to 0.51 mm at Site 4 and negligible silt-clay contents (Table 5.1).

Table 5.1 Beach elevation, slope, and sediment of *T. tridentatus* nests in Tsuyazaki, Fukuoka, Japan

Site	Beach elevation (m)	Beach slope (°)	Sediment						
			Grain composition †	Median diameter (mm)	Grain size sorting (φ)	Silt-clay content (%)	Skewness (φ)	Gravel (%)
Site 1	TP+0.57 ± 0.06	8.34 ± 2.03	FS (76.8 %) / MS (22.8 %)	0.19 ± 0.01	0.58 ± 0.04	0.09 ± 0.08	-0.38 ± 0.03	0.09 ± 0.15
Site 2a	TP+0.74 ± 0.08	6.00 ± 2.16	FS (61.4 %) / MS (31.2 %)	0.23 ± 0.05	0.96 ± 0.49	0.18 ± 0.24	-3.20 ± 4.12	6.05 ± 9.10
Site 2b	TP+0.12 ± 0.03	4.63 ± 0.55	MS (72.5 %) / FS (25.8 %)	0.31 ± 0.01	0.71 ± 0.01	0.03 ± 0.05	0.31 ± 0.11	0.04 ± 0.05
Site 3	TP+0.70 ± 0.04	7.55 ± 0.66	FS (63.1 %) / MS (32.2 %)	0.22 ± 0.04	0.85 ± 0.35	0.06 ± 0.08	-2.19 ± 3.05	3.15 ± 5.65
Site 4	TP+0.53 ± 0.20	7.43 ± 2.74	MS (58.6%) / CS (17.5 %) / FS (14.2 %)	0.51 ± 0.05	1.31 ± 0.21	0.20 ± 0.07	-0.96 ± 3.25	8.40 ± 2.92

Abbreviations: CS, coarse sand; MS, medium sand; FS, fine sand; T.P., Tokyo Peil: the average sea level in Tokyo Bay.

† The grain sizes that predominated at each site are presented.

5.3.2 GLM Analysis of Nest/Egg Occurrence and Physical Parameters

The results of the analysis of nest/egg occurrence and the physical parameters using generalised linear models (GLMs) are shown in Table 5.2. The beach elevation and the slope significantly influenced the nest/egg occurrence, whereas no significant contributions were found with sedimentary parameters except for the percentage of gravel (p<0.05) (Table 5.2). Thus, beach elevation, slope, and the percentage of gravel were selected to predict the nest/egg occurrence. The changes in the predicted values of the nest/egg occurrence with beach elevation, slope, and the percentage of gravel are shown in Figure 5.3. The highest nest/egg occurrence was estimated between T.P. +0.5 m and T.P. +0.9 m in beach elevation, in which the probability of nesting was 89 - 92% (Figure 5.3a). The highest nest/egg occurrence was predicted for the beach slope between 6.0° and 10.2° with 65 - 70% probabilities (Figure 5.3b). For the percentage of gravel, the highest nest/egg occurrence was predicted between 8.5 and 11.2 %, with 79 % probabilities (Figure 5.3c).

Table 5.2 Generalised linear model analysis results for the relationship between nest/egg occurrence and physical parameters.

Explanatory variable	Estimate	Standard error	*Z* value	Probability (>|*z*|)
Elevation	15.524	3.205	4.844	1.27e-06 ***
squared	-11.287	2.713	-4.161	3.17e-05 ***
intercept	-2.808	0.772	-3.639	0.000274 ***
Slope	0.892	0.382	2.332	0.020 *
squared	-0.055	0.027	-2.002	0.045 *
intercept	-2.756	1.272	-2.167	0.030 *
Median diameter	-8.062	7.199	-1.220	0.263
squared	7.768	8.542	0.909	0.363
intercept	2.044	1.324	1.544	0.123
Grain size-sorting	-1.654	3.839	-0.431	0.667
squared	0.655	1.717	0.381	0.703
intercept	1.278	1.904	0.671	0.502
Silt-clay content	-14.654	8.394	-1.756	0.081
squared	29.187	27.371	1.066	0.286
intercept	1.732	0.629	2.456	0.00586 **
Skewness	0.029	0.118	0.248	0.804
squared	0.009	0.147	0.616	0.538
intercept	0.330	0.265	1.245	0.213
% gravel	0.224	0.130	1.979	0.048 *
squared	-0.012	0.006	-2.157	0.031 *
intercept	0.148	0.309	0.480	0.631

***$p<0.001$, **$p<0.01$, and *$p<0.05$

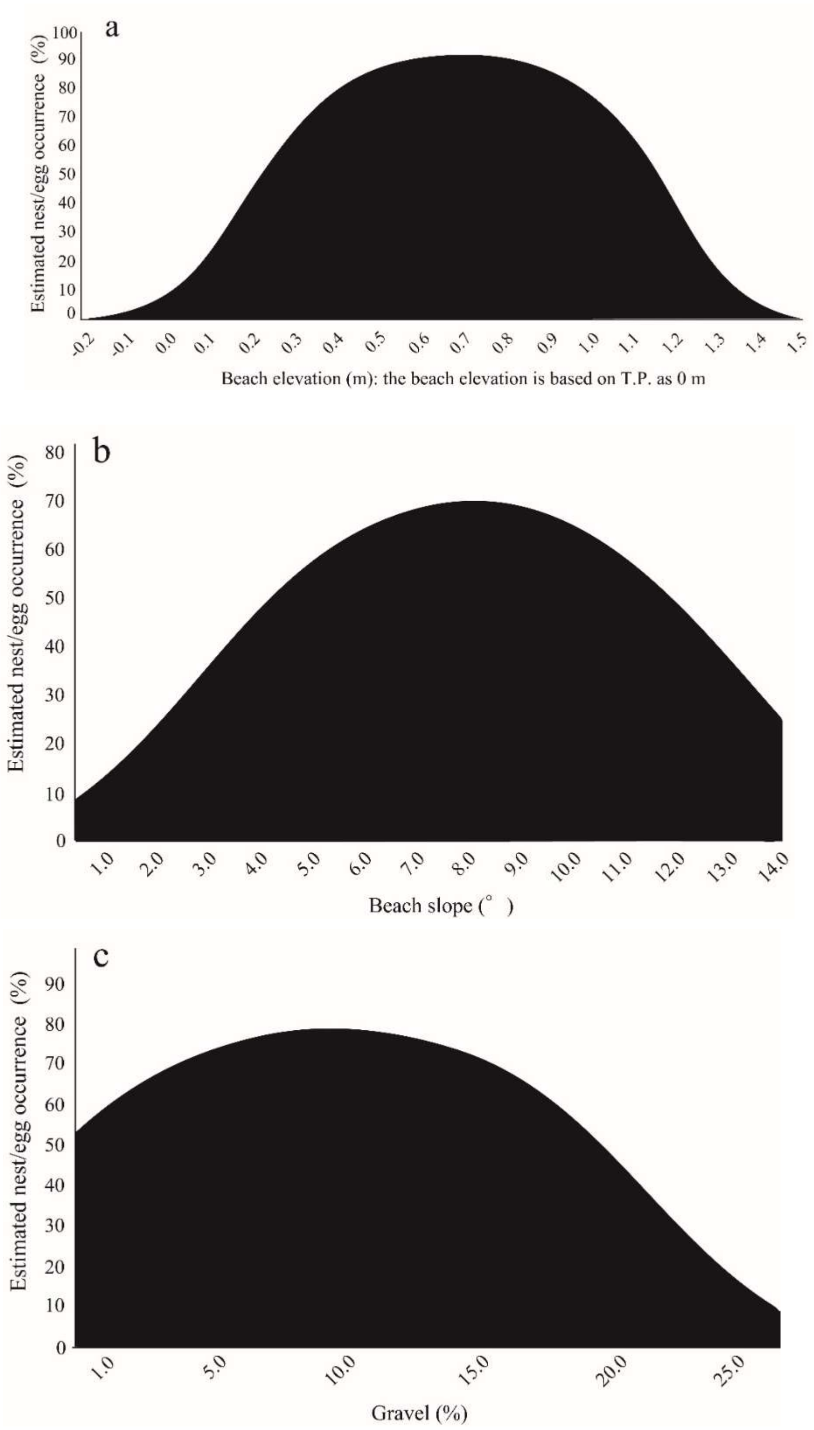


Figure 5.3 Nest/egg occurrence predicted by GLMs with a) beach elevation, b) beach slope and c) percentage of gravel.

5.3.3 Habitat Suitability Modeling and Mapping for Spawning Grounds

The GLM results showed that the beach elevation, slope, and percentage of gravel were appropriate variables for predicting suitable nesting grounds (p<0.05) (Table 5.2 and Figure 5.3). For the Maxent analysis, the beach elevation and slope were used, and the percentage of gravel was excluded. There was no correlation between the beach elevation and slope (r=0.32, Pearson's correlation coefficient), suggesting that these variables were independent (i.e., one variable does not affect the other, making it possible to construct a model by combining each independent variable). Thus, three models, namely, Model 1 (beach elevation), Model 2 (beach slope) and Model 3 (the combination of the beach elevation and slope), were constructed at Site 2a as "Training data", using Maxent to confirm the contribution of each variable to the habitat suitability. These models were further extrapolated to Sites 1, 2b, 3 and 4 as "Test data" to verify the accuracy of the models. Model 2 (slope) had an AUC value <0.7, meaning the model's accuracy was poor. Even though Model 3 (the combination of elevation and slope) showed an AUC value >0.7, the model had a lower AUC than Model 1 (elevation), indicating that constructing a model with beach elevation alone was more accurate than the combination of elevation and slope. Model 1 (elevation) had 0.849 AUC (exceeding 0.7 and close to 0.9, which is suggestive of the high accuracy of the model) and high sensitivity (Table 5.3); the model was considered reliable. Thus, the beach elevation was selected as the most appropriate parameter to construct HSMs (habitat suitability models) for spawning sites.

Based on the best Maxent model, suitable spawning habitats were mapped using "Training data (Site 2a) " and "Test data (Sites 1, 2b, 3, and 4) " (Figure 5.4). The cut-off value that maximises sensitivity and specificity was 0.372 (Table 5.3). The nesting probability distribution with this cut-off value was converted into actual elevation, it was estimated that T.P. +0.51m was the threshold and the elevation zones higher than the threshold were the suitable habitats for nesting

(Figure 5.4). 74 % of the nests (32 in 43 nests) were in the suitable zone. The remaining 26 % of the nests (11 in 43 nests) were in the unsuitable zone (Figure 5.5). Based on this model, the remaining area of suitable spawning ground was 476 m^2 (24 % of the beach at Sites 1 - 4). Larger suitable nesting areas remained at Site 1 and Site 2a than at the other sites (Figure 5.6).

Table 5.3 Summary of Maxent results.

Model Name	AUC	Cut-off value	Training Data Sensitivity	Test Data Sensitivity
Model 1 *, **	0.849	0.372	Site 2a: 1.000	Site 1: 0.857
(Elevation)				Site 2b: 0.000
				Site 3: 1.000
				Site 4: 0.615
Model 2	0.653	0.561	Site 2a: 0.111	Site 1: 0.857
(Slope)				Site 2b: 0.000
				Site 3: 0.444
				Site 4: 0.385
Model 3	0.845	0.373	Site2a: 1.000	Site 1: 0.857
(Elevation + Slope)				Site 2b: 0.000
				Site 3: 1.000
				Site 4: 0.615

* The highest sensitivity model

** The highest AUC model

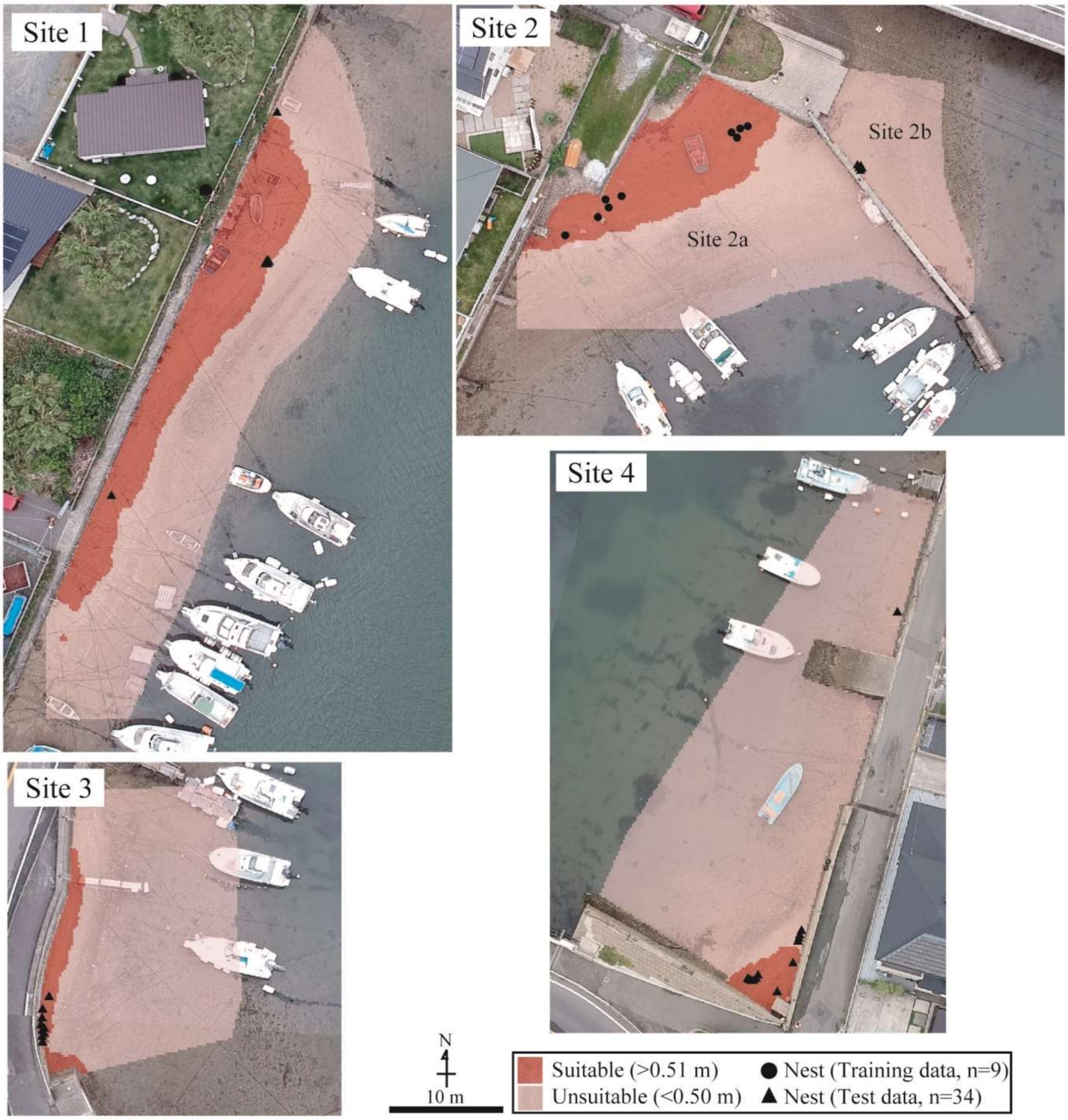


Figure 5.4 Estimated spawning suitability map for *Tachypleus tridentatus* in Tsuyazaki, Fukuoka, Japan.

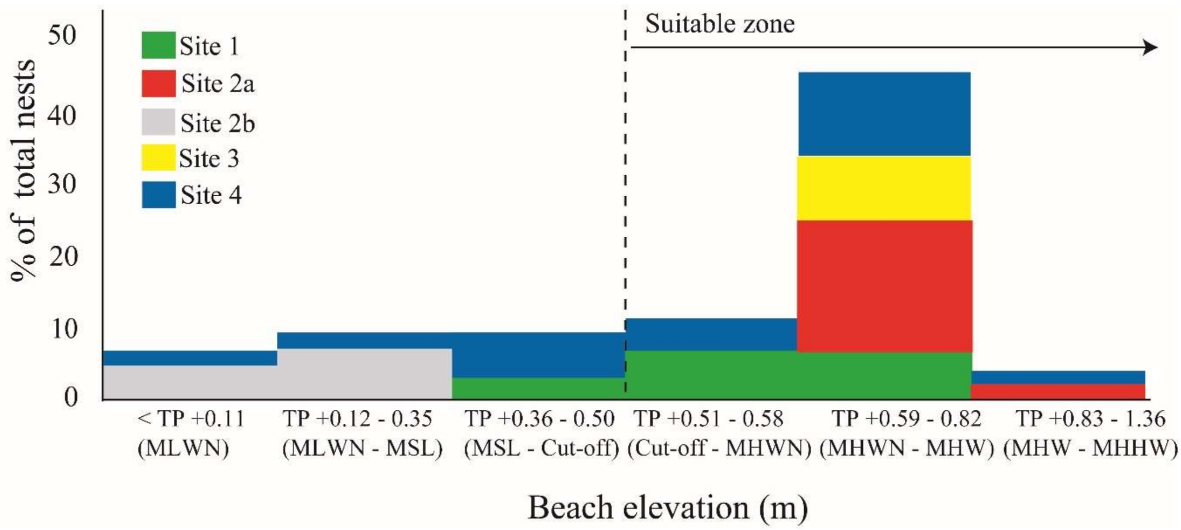


Figure 5.5 Percentage of total nests based on field sampling. The elevation zone above 0.51m (Cut-off) is estimated to be suitable spawning habitat. The abbreviations are as follows: MHHW (Mean Higher High Water), MHW (Mean High Water), MHWN (Mean High Water Neap), MSL (Mean Sea Level), MLWN (Mean Low Water Neap).

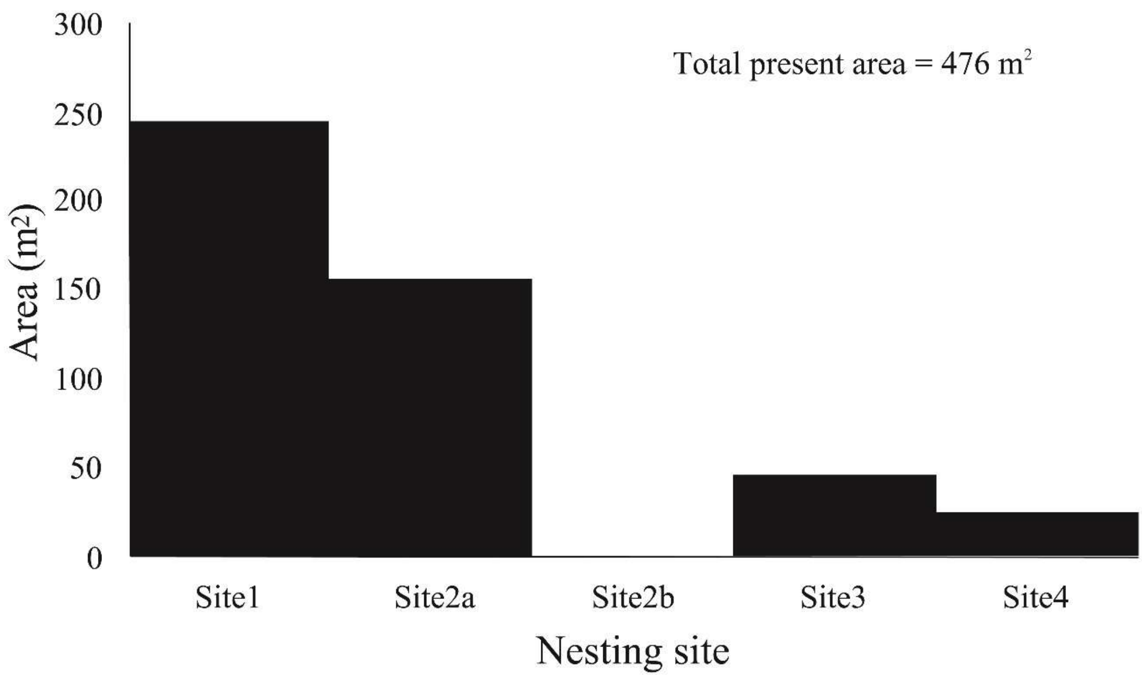


Figure 5.6 Estimated present suitable spawning area in Tsuyazaki, Fukuoka, Japan.

5.4 Discussion

5.4.1 Estimation of Suitable Spawning Habitat

This chapter attempted to predict suitable spawning habitats for the first time in *T. tridentatus* using scientific models based on the nest/occurrence and physical parameters of known spawning sites in Tsuyazaki Cove, Fukuoka. The suitable spawning zone estimated by Maxent using Model 1 (beach elevation) was from MHHW to the cut-off (around MHWN), and 74 % of the nests fitted into the suitable zone (Figures 5.4 and 5.5). The estimated zone is consistent with previous studies showing that all almost *T. tridentatus* nests were found between the MHHW and MHWN. In Japan, almost all nests in the significant *T. tridentatus* spawning habitats in Kyushu were found within the same tidal range; 86 % (378 of 439 nests) in Imazu, Kafuri, Tsuyazaki and Sone habitats of Fukuoka (Wakamiya, 1989), 97 % (400 of 414 nests) in Imari, Saga (Sekiguchi, 1999). Although comparative studies are limited in other Asian countries due to the lack of *T. tridentatus* spawning data (Wang et al., 2020), 75 % of the nests (51 in 68 nests) were detected between the same tidal range in Malaysia (Mohamad et al., 2019). Thus, it can be concluded that although the sampling size in the study of this chapter was small (n=43) due to the rapid decline of the horseshoe crabs in the area (Itaya et al., 2019b; Itaya et al., 2022b), the estimated suitable zone reflects the actual spawning preference of the species.

By contrast, some nests were in unsuitable areas (i.e., below the cut-off) (Figures 5.4 and 5.5). *T. tridentatus* spawning beaches can gain oxygen when exposed to the air at low tide, and the oxygen in the sand is dissolved into the pore water when immersed in seawater at high tide and eventually absorbed by the eggs. Therefore, *T. tridentatus* eggs develop better if they are completely removed from seawater at a certain frequency rather than constantly immersed in seawater (Sekiguchi, 1999). Such moderate ebb and flow do not occur below MHWN, so *T. tridentatus* egg survival is the poorest at the elevation lower than MHWN due to the lack of air exposure time (Itaya et al., 2022a). A similar phenomenon was evident in the Atlantic horseshoe crab (*Limulus polyphemus*). *L. polyphemus* eggs decayed due to insufficient oxygen and increased hydrogen sulfide at elevations lower than MHWN (Penn & Brockmann, 1994). Therefore, it is likely that horseshoe crab egg survival would be low in the area estimated to be unsuitable by the model, even though some nests were observed. It is known that *T. tridentatus* may lay eggs at lower tidal zones with limited suitable spawning grounds because of coastal development (Sekiguchi, 1999; Itaya et al., 2019a; Kwan et al., 2022), indicating some nests can occur in the unsuitable tidal zone. It is possible that the area estimated as unsuitable is not an underestimation of spawning grounds by the model but rather a prediction of the tidal levels at which successful egg development depends.

Although Maxent is a highly effective modelling tool for understanding the potential distribution of threatened species (Ishihama, 2017), Warren & Seifert (2011) warn that models using only occurrence data often risk overfitting with training data. Therefore, care should be taken when interpreting the results. In this chapter, the area around MHHW was also included as suitable by the Maxent analysis (Figures 5.4 and 5.5). However, *T. tridentatus* appears to avoid between MHHW and the upper part of MHW, as reported by Wakamiya (1989) & Sekiguchi (1999). The former found 0 in 439 nests, and the latter observed 1 in 414 nests between MHHW and the above MHW. Penn & Brockmann (1994) speculated that *L. polyphemus* avoids spawning in the dry and hot environment of the upper beach. Nelson et al. (2019) also mentioned that the coastal horseshoe crab (*Tachypleus gigas*) nests around MHHW were prone to extreme heat exposures, causing desiccation. In a laboratory experiment with *T. gigas*, eggs placed in the sand had a poor development rate when the eggs were not submerged in seawater for more than a week, which caused desiccation (Faizul et al., 2013). In Tsuyazaki Cove, while the MHW-MLWN zone was immersed in seawater at least once a day, there were three times when the beach area around the MHHW zone was not submerged in the seawater for more than one week during the 49-day incubation period, causing desiccation and a lack of available oxygen for the eggs, resulting in a poor egg development rate (Itaya et al., 2022a). It is, therefore, likely that the area between MHHW and the above MHW predicted by Maxent as suitable is an overestimate in terms of egg survival.

Araújo & New (2007) proposed an "ensemble forecast" technique by combining the predictions of multiple models to reduce the risk of making predictions far from the truth. In GLM analysis, nest/egg occurrence was the highest between T.P. +0.5 m and T.P. +0.9 m (Figure 5.3a), including the actual distribution of the highest nest/egg occurrence zone (T.P. +0.58 m - T.P. +0.82 m; Figure 5.5). Therefore, considering the evaluation by GLM as a complement to the overestimation of Maxent, it is reasonably suggested that the most suitable zone for spawning in the study of this chapter is from T.P. +0.5 m (the cut-off line) to T.P. +0.9 m (just above MHW). Penn & Brockmann (1994) concluded that *L. polyphemus* maximised their egg development rate by selecting the middle part of the spawning beach, avoiding the dry and hot environment in the upper beach and the oxygen-deficient condition in the lower beach. The same can be said for *T. tridentatus*. In the middle part of the beach (T.P. +0.5 m and T.P. +0.9 m), the saturation and dehydration frequency are optimised by the relationship between the tidal movement and the nest locations during the egg development period. This, in turn, maintains moderate water content supplying sufficient oxygen for the eggs and, as a result, the survival rate of the *T. tridentatus* eggs is the highest at the middle part of the spawning beach (Itaya et al., 2022a). Thus, it can be concluded that *T. tridentatus* maximise the survival rate of their eggs by selecting the middle part of the spawning beach.

5.4.2 Application of the Current Approach to Other Horseshoe Crab Spawning Habitats

The study of this chapter suggested that beach elevation relative to tidal range is an adequate parameter for predicting the suitable spawning zone for *T. tridentatus* (Table 5.3). The estimated suitable spawning zone was highly compatible with other *T. tridentatus* habitats with a wide range of grain compositions - fine to medium sand in this chapter, medium to coarse sand in Fukuoka (Wakamiya, 1989), gravelly sand in Imari (Botton et al., 1996), and fine to medium sand in Malaysia (Mohamad et al., 2019 - indicating that the beach elevation model (or tidal level model) can be applied to other *T. tridentatus* spawning grounds. It should be added to avoid misunderstanding that the direct application of the suitable spawning elevation range (T.P. +0.5 m - T.P. +0.9 m) delineated in this chapter to other *T. tridentatus* spawning habitats is meaningless. Given the inherent variability in tidal ranges across diverse geographical areas, a model akin to the one employed in this chapter, which utilises beach elevation as an explanatory variable, implies that the "elevation" characterising a suitable spawning zone should dynamically adjust to the unique tidal range of each specific region. Consequently, when applying the methodology of this chapter to *T. tridentatus* spawning grounds in distinct locales, it is imperative to calculate the relative beach elevation (or tidal level) based on the specific tidal ranges inherent to each area.

While the model demonstrated strong applicability to *T. tridentatus* spawning habitats, its direct application to other horseshoe crab species (e.g., *Limulus polyphemus*, *Tachypleus gigas*, and *Carcinoscorpius rotundicauda*) raises doubts. This scepticism arises from these species spawning in environments distinct from that of *T. tridentatus*. Several factors, such as beach elevation relative to the tidal range and sediment types, are considered as their spawning requirements (e.g., Penn & Brockmann, 1994; Nelson et al., 2016; Nelson et al., 2019; Zauki et al., 2019; Kwan et al., 2022). For example, *C. rotundicauda* spawns on muddy sand beaches

(Bottn et al., 1988; Sekiguchi, 1999), and their nests are mainly located around low tide marks (Zauki et al., 2019; Zauki, personal communication). Still, the relative relationship between beach elevation and tidal range is one of the critical factors determining egg survival in horseshoe crabs (Penn & Brockmann, 1994; Itaya et al., 2022a), and the beach elevation relative to tidal range can be used as an explanatory variable in the analysis.

Maxent may overestimate the habitat suitability because it uses only the "occurrence" data (Figure 5.4). Nevertheless, it can be adjusted by combining it with the GLM analysis result, as discussed above. Thus, combining multiple models is recommended to compensate for each model's advantages and disadvantages and to make the model's predictions more accurate when applying the current approach to other species of horseshoe crabs.

5.4.3 Limitations of the Study

Although the estimated suitable spawning zone was highly compatible with other *T. tridentatus* habitats, the HSM constructed in this chapter did not show extremely high predictive accuracy (AUC=0.849 in Model 1, which is less than 0.9 AUC; Table 5.3). The gravel content significantly contributed to nest/egg occurrence in the GLM analysis (Table 5.2). It was reported that the sediments of the spawning ground from MHHW to MLWN are not a controlling factor for egg survival in Tsuyazaki (Itaya et al., 2022a). However, it was revealed that the sediment properties, such as the grain composition, grain sizes and gravel content, affect the compressive strength of beaches and consequently affecting sediment permeability and availability of oxygen to the eggs in other regions (Penn & Brockmann, 1994; Nelson et al., 2016; Botton et al., 2018; Nelson et al., 2019). Therefore, sediment can be one of the influential variables in estimating suitable spawning grounds.

Despite the significant contribution of the gravel content to nest/egg occurrence in the GLM analysis (Table 5.2), it could not be applied to the Maxent. Recently, species distribution models based on statistical methods such as Maxent have come to be used in various fields of ecology due to the accumulation of biological distribution data, the methodological development for estimation and the high accessibility of environmental information data based on remote sensing (Ishihama, 2017). In most cases, open-source data of environmental information such as vegetation cover and land use, which are available on the internet and therefore quickly obtained, have been used (e.g., Arakida & Mitsuhashi, 2008; Ozaki et al., 2008; Kusano, 2016).

In the case of environmental information such as local beach sediments for which no open-source data are available, the investigators must collect these data for the Maxent analysis, as in this chapter. Maxent requires raster data, so sediment distribution must be rasterised as in other parameters such as beach elevation. However, it was impractical for the sedimentary parameters. Koyama et al. (2020) applied a habitat suitability model for *T. tridentatus* juveniles using Maxent, and sedimentary parameters, one of the factors influencing the distribution of juveniles, were not included for the same reason. Furthermore, Iyooka et al. (2015) pointed out that since remote sensing is still a developing technology, it is expected that further developments will improve the ability to estimate habitat factors, such as sediment, which influence the target species' distribution. For example, a recently developed technology, a multispectral unmanned aerial vehicle, was used to estimate the spatial distribution of the silt-clay content of the *T. tridentatus* juvenile habitat (Yamamoto et al., 2022). Thus, it is expected that as the technology progresses, sediment distribution on the spawning beach can be rasterised for Maxent in the future.

5.4.4 Urgency to Protect Spawning Beaches and Recommendations

The remaining suitable spawning habitat was estimated to be only 476 m^2 (24 % of the available beach at Sites 1 - 4) in the study area (Figure 5.6). Sites 1, 2 and 3 were originally part of one continuous sandy beach. However, it has been fragmented due to coastal developments, and now only this tiny area remains (Itaya et al., 2019b; Itaya et al., 2022b). Therefore, it can be strongly recommended that the conservation and reconstruction of spawning grounds be a high priority at the study site.

In Tsuyazaki, the loss/deterioration of the spawning habitat appears to have caused not only a decrease in the number of spawning adults but also a decline in the juvenile population. As tri-spine horseshoe crabs use different coastal environments -sandy mudflats for the juveniles, shallow coastal water for the adults, and sandy beaches for spawning (Sekiguchi, 1999)- multiple causes can trigger local declines, such as juvenile habitat degradation/loss, adult habitat degradation/loss, spawning ground degradation/loss, or any combination of these. *T. tridentatus* is not commercially harvested in Japan (Sekiguchi, 1989), thus overfishing is not considered an issue. Annual juvenile records from 2003 to 2019 using a standard method show a steady decline in juvenile numbers in the area; on average, 97 juveniles were recorded per survey between 2003 and 2006 (Wada et al., 2008), whereas the 2007-2016 surveys averaged 24 individuals per survey (Itaya & Shuuno, unpublished data) and an average of only 7 juveniles per survey from 2018 to 2019 (Koyama et al., 2020). The suitable nursery habitat in Tsuyazaki was estimated to be 61,000 m^2 (out of the investigated area of 195,000 m^2) (Koyama et al., 2020). There have been no landfills or development works within the juvenile habitat between 2003 and 2019, hence no further habitat destruction in the area. For the adult horseshoe crab environment, no construction works have occurred along the coast except for around spawning sites (i.e., the entrance of the cove) during the same period in the area (Itaya, personal observation). As the juvenile and adult habitats have not changed since 2003, the degradation of the spawning habitat in recent years is most likely responsible for the decrease in juveniles.

One of the practical actions is to ensure that there is no further deterioration in spawning beaches in Tsuyazaki Cove. Beach protection structures such as seawalls and bulkheads built within the intertidal zone reduce the spawning habitat for horseshoe crabs (Botton et al., 1988; Jackson et al., 2015). These artificial shoreline structures also disrupt the natural corridors of longshore transport of sediments (Jackson et al., 2015). In Japan, such structures have affected most of the available spawning habitats of *T. tridentatus* (Sekiguchi, 1989; Botton, 2001). The present study area had large beaches until the early 1970s, but these have been fragmented into the current sites due to coastal development, which continued until the 2000s (Itaya et al., 2019b; Itaya et al., 2022b). As the spawning numbers have declined to <10 % in Tsuyazaki following the construction of the yacht harbour, breakwaters and surrounding artificial structures at the bay mouth in the early 2000s (Itaya et al., 2019b; Itaya et al., 2022b), the removal of these structures or changes that allows sand to flow to the spawning ground should be considered.

In addition, beach nourishment (i.e., supplying sand manually to the spawning ground) should be considered (Itaya et al., 2022b), and this may be an ecologically preferable strategy (Botton, 2001). Beach nourishment for spawning grounds of *T. tridentatus* has been carried out in some places (Seino et al., 2000; Ohtsubo et al., 2005; Hsieh & Chen, 2009). Ohtsubo et al. (2005) reported that spawning activity increased after beach nourishment in Imazu, Fukuoka, compared to un-nourished areas. The sand that had been supplied to the spawning ground before the coastal development now has been trapped and accumulated around the bay mouth after the development (Itaya et al., 2019b; Itaya et al., 2022b), and the accumulated sand's properties (fine sand with a median diameter of 0.17 mm and negligible silt-clay content) are almost identical to Sites 1, 2 and 3 (Itaya, unpublished data). Therefore, the sand accumulated around the bay mouth could be used for beach nourishment in Tsuyazaki Cove.

5.5 Conclusion

The suitable spawning habitat of *T. tridentatus* in Tsuyazaki Cove was estimated with modelling using nest/egg occurrence and the physical parameters. It was found that beach elevation relative to tidal range is the most effective parameter for modelling suitable spawning habitats in the cove, which can then be mapped, making it possible to establish the habitat status of coastal areas suitable for spawning. This approach can be extended to other *T. tridentatus* spawning sites. Consequently, it provides a valuable basis for planning effective conservation strategies for this globally endangered species. One of the notable advantages of this approach is its ability to evaluate the suitability of spawning habitats for *T. tridentatus* readily. By mapping the analytical data set, researchers, government agencies, and the public can quickly grasp which habitats should be prioritised for conservation efforts. This method can be a powerful decision-making tool in conservation initiatives. However, it should be noted that continuous improvement is necessary, especially with the integration of new remote-sensing technologies.

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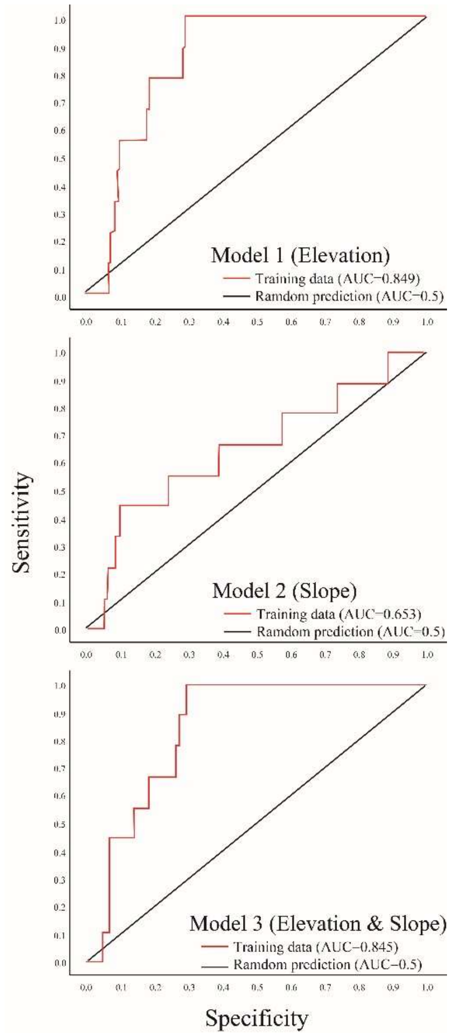
Appendices



Appendix 5.I The Area Under Curve (AUC) of the Receiver Operating Characteristic (ROC) curve for *T. tridentatus* suitable spawning habitat models. The vertical axis shows the proportion of "presence" or "occurrence" data that are correctly predicted (sensitivity). The horizontal axis shows the proportion of "absence" or "non-occurrence" data that are incorrectly predicted to be present (false positives or specificity). "Absence" or "non-occurrence" data is randomly selected from background data in Maxent. The area under the ROC curve is the AUC; the closer the AUC is to 1, the better the models. The AUCs for each model were 0.849 for Model 1 (beach elevation), 0.653 for Model 2 (beach slope) and 0.845 for Model 3 (beach elevation & slope), with a random prediction AUC of 0.5.

Chapter 6

Overall Conclusions and Recommendations

6.1 Conclusions

Prior to the commencement of the study, a predefined set of research objectives was established to provide a clear framework and ensure the successful attainment of these objectives. The primary objective of the published research was to investigate the underlying factors contributing to the significant decline in *Tachypleus tridentatus* spawning numbers in Tsuyazaki Cove.

The first objective was to examine the historical spawning habitat changes of *T. tridentatus* in Tsuyazaki Cove and assess their implications for the decline of the species. This was undertaken by collecting spawning habitat information from 1948 to 2010 and field spawning surveys from 2005 to 2013 (Chapter 2). The second objective involved a preliminary investigation to examine the spawning site selection of *T. tridentatus* and evaluate the potential impact of coastal development in the study area (Chapter 3). The third objective focused on elucidating the relationship between the egg survival rate and the environmental factors that determine suitable nesting zones and to assess the specific effects of coastal development on the survival of *T. tridentatus* eggs (Chapter 4). Finally, scientific models were developed and applied to evaluate the suitability of spawning sites in Tsuyazaki Cove. In addition, the remaining suitable spawning habitats in the area were mapped based on the modelling results (Chapter 5).

The conclusions drawn from the results of this research study are as follows:

In Chapter 2, historical spawning habitat changes of *T. tridentatus* in Tsuyazaki Cove were assessed by analysing aerial photographs from 1948 to 2010. This data was compared with interviews with residents and field monitoring of *T. tridentatus* spawning pairs. The findings from this analysis provided insight into the factors leading to the decline of *T. tridentatus*. The findings indicated that coastal development, particularly the modification of the bay mouth and the disruption of sediment transport routes had negatively impacted the spawning sandy beaches. The lack of ecological knowledge and absence of formal protection measures for the species further contributed to the decline of spawning sites and the disruption of natural processes involved in beach formation. This chapter highlighted the importance of tracing habitat changes over time and comparing them to current conditions for gaining an understanding the impact of coastal development. This approach also provided valuable insights into the causal relationship between the *T. tridentatus* population decline and the negative effects of human activities such as landfilling.

Chapter 3 reported on an investigation to explore the spawning site selection of *T. tridentatus* and assessed the underlying impact of coastal development by examining the physical characteristics of the spawning beach. It was found that the alteration of the beach shape had occurred through repeated landfills. Landfills had profoundly impacted the availability of sediments for the spawning grounds of *T. tridentatus*. A well-preserved original beach shape emerged as a potential sanctuary with various nesting elevations. On the other hand, some nests were located at lower elevations, previously thought unpreferable. Consequently, it was concluded that conservation initiatives should prioritise protecting and restoring natural beach features to help the availability of suitable spawning habitats for this critically endangered species.

In Chapter 4, *T. tridentatus* eggs were translocated to different intertidal elevations in order to examine the relationship between egg survival rate and environmental factors with the aim of identifying the most suitable nesting zone for egg viability. The results yielded significant insights into the influence of physical characteristics of the spawning ground on egg survival. Beach elevation relative to the tidal range was found to play a critical role, with the mid intertidal zone being the most suitable for egg survival. This zone provided optimal conditions for the full development of eggs: in particular, appropriate air exposure time, adequate water content, and suitably sized sediments. The duration of beach exposure to air and water content, influenced by beach elevation, strongly affected egg survival. Higher and lower intertidal zones, with excessive or insufficient air exposure, negatively impacted egg survival. The dynamic pattern of moderate saturation and dehydration in the mid intertidal zone proved crucial for egg survival. It was concluded that protecting mid intertidal zones as suitable spawning areas was of great importance for managing and conserving this globally endangered species. Additionally, it was suggested that the translocation of eggs from marginal zones to more suitable spawning zones could be a potential strategy for population recovery. Relocating eggs to environments that provide more favourable nesting conditions for egg development and survival could enhance the species' reproductive success and overall resilience to environmental change.

In Chapter 5, scientific modelling approaches were employed to evaluate the suitability of critical spawning sites in Tsuyazaki Cove and to map the remaining suitable spawning habitats. The model successfully estimated the suitable spawning habitat for *T. tridentatus* using analysed nest/egg occurrence data and physical parameters. The findings emphasised the significant role of beach elevation relative to the tidal range as the primary determinant of suitable spawning habitats. The study revealed that the remaining suitable spawning habitat in the area was limited to 476 m^2, which represented only 24% of the beach available for nesting. It was concluded that successive coastal development had fragmented the once-connected spawning beaches, leaving these as small and isolated sites less suitable for spawning.

In summary, the findings of this published research provided valuable insights into the impact of coastal development on the decline of the local *T. tridentatus* population in Tsuyazaki Cove. Chapter 2 revealed that the extensive fragmentation and degradation of spawning beaches, resulting from coastal development over time, likely contributed to the population decline. Chapter 3 emphasised the importance of preserving the original beach shape, which offers diverse nesting elevations. However, in degraded spawning beaches affected by coastal development, *T. tridentatus* pairs were observed laying eggs at lower elevations, previously considered unsuitable. Chapter 4 demonstrated that the mid intertidal zone exhibited the highest egg survival, with significantly lower survival rates observed at lower elevations. Chapter 5 further supported the significance of preserving the original beach shape as the estimated suitable spawning habitat based on scientific models that aligned with egg survivability. It is essential to highlight that this research provided valuable perspectives for the local *T. tridentatus* population and enhanced our understanding of the species' spawning ecology, which can apply to other *T. tridentatus* populations facing extinction worldwide. These comprehensive efforts have shed light on the causal relationships between coastal development and the degradation of spawning habitats, unveiling a factor resulting in the rapid decline of the *T. tridentatus* population in Tsuyazaki. Therefore, prioritising the conservation and restoration of the remaining spawning grounds in the study site is strongly recommended.

6.2 Recommendations

Considering the deterioration of the spawning ground and the drastic decline of the *T. tridentatus* population in Tsuyazaki Cove, it can be described as a state of near extinction where that the natural recovery of the population would not be possible under the current conditions (Itaya et al., 2019; Itaya et al., 2022b). With such a reduced population size, the local population could easily be driven to extinction by natural phenomena such as decreased numbers due to natural disasters, genetic drift, and inbreeding (Itaya et al., 2019). Given the current situation, the extinction of the horseshoe crab in Tsuyazaki Cove may be inevitable. Nevertheless, the following suggestions are proposed for the conservation of the local population of horseshoe crabs.

First and foremost, is ensuring that no further deterioration of the remaining spawning beaches occurs. This requires the protection of the remaining suitable spawning zone (i.e., beach elevation higher than T.P. +0.51 m), which is essential within the existing spawning area (Itaya et al., 2023). Also, it may be necessary to translocate eggs laid in the unsuitable zone to the more suitable zone to increase their survival rate (Itaya et al., 2022a). However, these conservation measures alone are insufficient in Tsuyazaki Cove, where almost all of the sand, once carried by currents into the cove from the open sea and forming the spawning grounds, has been lost due to coastal development and beach erosion (Itaya et al., 2019; Itaya et al., 2022b). Therefore, a variety of conservation measures need to be considered.

Secondly, it is necessary to restore the sand supply cycle to the spawning grounds. This will require rethinking how the existing beach infrastructure that includes the yacht harbour, breakwaters and beach protection structures in the intertidal zone have a detrimental effect on horseshoe crab spawning habitats. For example, they cause a reduction in suitable areas for spawning and disrupt the natural sediment movement along the coast (Botton et al., 1988; Jackson et al., 2015). This can be remedied by implementing changes that facilitate the flow of sand to the beaches, making spawning more likely. Coastal development in the study area has caused the once expansive beaches to become fragmented into smaller sites. This fragmentation, along with the construction of infrastructure in the cove, has resulted in a significant decrease in spawning numbers in Tsuyazaki (Itaya et al., 2019; Itaya et al., 2022b). Therefore, it is essential to consider implementing measures to facilitate the natural flow of sand to the spawning grounds.

As the yacht harbour is likely to be most responsible for blocking the inflow of sand from the open sea (Itaya et al., 2019; Itaya et al., 2022b), it is recommended that consideration be given to the possible reconstruction of the harbour with a floor raised above the sea level so that tidal currents would pass it below (Itaya et al., 2022b).

Also, considering the significant decline in spawning pairs since 2009 in particular (Itaya et al., 2019; Itaya et al., 2022b), removing the breakwater constructed in 2006 to block the only entrance of the cove is desirable. In their study, Wada et al. (2010) raised apprehensions regarding the impact of bay mouth blockage caused by the breakwater on the spawning grounds and breeding adult *T. tridentatus* in the area. Additionally, they emphasised the necessity of investigating the causal relationship between the rapid decline of *T. tridentatus* and the construction of these artificial features. Since this research study has identified some causal relationships between coastal developments and the reduction of *T. tridentatus* in the area, expeditious implementation of these conservation measures is recommended.

Within ecological considerations, beach nourishment is another favourable approach that has proven beneficial for spawning activity (Botton, 2001). For example, Ohtsubo et al. (2005) found that nourished areas exhibited higher spawning activity than non-nourished areas, highlighting the positive impact of beach nourishment for promoting spawning in Imazu, Fukuoka. Due to the insufficient sand supply in the spawning ground, manual intervention is probably required to restore the remaining spawning ground in the study area. To ensure effective beach nourishment in Tsuyazaki Cove, it is crucial to consider the dynamic nature of drifting sand, as recommended by Ishikawa et al. (2015). In this context, the maintenance of artificially nourished spawning habitats necessitates the transportation of sand from the divided sandy beach caused by development to the existing spawning ground. Notably, the current spawning sites (Sites 1, 2, and 3) and the sandy beach near the yacht harbour were initially connected as a continuous sandy beach (Itaya et al., 2019; Itaya et al., 2022b), and the sand which has accumulated around the yacht harbour after its construction exhibits similar properties to Sites 1, 2, and 3 (Itaya, unpublished data). Therefore, the sand accumulated around the yacht harbour area could be used for restoring the spawning ground.

Conservation regulations for horseshoe crabs and establishing marine reserves should also be considered. Although many essential spawning beaches, such as Delaware Bay in the U.S., have received protection (Botton, 2001), MPAs (Marine Protected Areas) and legislation are primarily unavailable and ineffective in most Asian counties (Wang et al., 2020). Also, although *T. tridentatus* is listed as an endangered species in the Red Data Book of Japan (Ito, 2014), simply relying on the species' listing on the Red Data alone does not provide sufficient protection. One approach is to use existing legal frameworks, such as the Cultural Heritage Protection Act and prefectural regulations, which could be suitable for safeguarding *T. tridentatus* habitats. Currently, Kasaoka (Okayama prefecture) and Imari (Saga prefecture) habitats are designated national natural monuments, and the Saijyo City (Ehime prefecture) spawning site is officially recognised as a prefectural natural monument. In Nagasaki prefecture, the species is protected under a prefectural regulation. However, no specific regulations exist for *T. tridentatus* habitats in Hiroshima, Yamaguchi, Oita, and Fukuoka prefectures (Ohtsuka et al., 2017). Therefore, there is an urgent need to implement legal regulations to protect *T. tridentatus* habitats in Tsuyazaki, Fukuoka.

Factors involved in the decline of horseshoe crabs are considered to be more complex than those discussed in this study. For example, as the dispersal capabilities of the hatchling larvae appear to be limited (Botton, 2001), the alternation of tidal currents involved with the artificial modification of habitats may inhibit the dispersion of larvae from reaching their nursing habitat (Maeda et al., 2000; Seino et al., 2000). Also, it should be noted that once a population is depleted, whatever the cause, it will take decades to recover due to the long time lag between egg deposition and adult recruitment (Botton, 2001). Given these complexities, understanding the multifaceted factors influencing horseshoe crab decline is imperative for effective conservation strategies.

Further studies are required to gain more insights into the impact of coastal development on horseshoe crab habitats in the area. For instance, understanding the alterations in tidal currents and other physical conditions due to coastal development is crucial for identifying the factors contributing to the decline of the local population and developing targeted conservation measures. Additionally, it is essential to investigate effective approaches for preserving and replenishing the sandy beaches that serve as suitable spawning sites. This will help prevent further degradation of their spawning grounds.

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