Studies on the ecology of small cetaceans in the Istanbul Strait using acoustical information

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Abstract

Growing human populations and economic activity have a negative impact to marine ecosystem, such as marine pollutions, overexploitations, extinction of species and following shrink of biodiversity. Small cetaceans, which belong to suborder Odontoceti, are one of the important mammals that include likely-to-be-extinct species because of human activities in the last decade. More than half of small cetacean species are listed as “Endangered” or “Data Deficient” in the International Union for Conservation of Nature (IUCN) Red List. For effective conservation, the knowledge of their basic ecology is necessary.

In the present study, I focused on the population observed in the Istanbul Strait. Three species are observed in this area; harbor porpoise *Phocoena phocoena*, bottlenose dolphin *Tursiops truncatus* and short-beaked common dolphin *Delphinus delphis*. All of them are threatened subspecies but their ecology based on long-term constant observation has not well documented. I applied passive acoustic monitoring (PAM) with new species discrimination and animal density estimation method to fit on the situation of the Istanbul Strait and obtained the ecological information. The PAM is now widely used for study on small cetaceans, but the species discrimination based on acoustic information is still challenging. I proposed the discrimination method between harbor porpoise and delphinids and evaluated the accuracy by model. Also, I proposed the animal density estimation method which combine the line transect distance sampling approach with point transect estimations in consideration of the misclassification rate of species.

Two-year continuous PAM was conducted by fixed acoustic tag (A-tag) in the middle of the strait in 2010–2011. I revealed that harbor porpoise were detected most in spring (March–May), and there are almost no detections in winter (December–February). On the other hands, delphinids were observed throughout the year during nighttime. The porpoises distinctively showed short inter-click intervals that imply the feeding behavior in spring, suggesting that they were foraging the migrating pelagic fish in spring. It was supported by the previously reported stomach contents of the stranded porpoises which were found in the Marmara Sea and the Turkish coast of the Black Sea.

The animal density and distribution in whole strait were estimated by towed PAM in 2013. Animal density and distribution are one of the most fundamental information for conservation. In the Istanbul Strait, only the distribution of bottlenose dolphin has been available so far. I applied proposed density estimation method which can cover wider range of area by line transect approach based on acoustic cue based point transect distance sampling estimation. Also, I considered misclassification rate of species in estimator. The density was estimated as average of all seasons, it was 0.013 individuals km^{-2} (CV = 0.612) for harbor porpoise and 0.033 individuals km^{-2} (CV = 0.823) for delphinids, respectively. The distribution of harbor porpoise tended to be dispersed in the strait, but that of delphinids tended to be localized in north entrance to the Black Sea. The 50% kernel core area did not overlap between harbor porpoise and delphinids, suggesting the core habitat separation.

I developed the PAM methodology that is suitable for the small cetacean species appeared in the Istanbul Strait, and obtained the ecological information in present study. The results revealed the difference of seasonal behavior, distribution and animal density between harbor porpoise and delphinids within the strait. It implies the effectiveness of PAM also in the multi-species-observed noisy area, and the importance to consider the interspecific difference of ecology for effective conservation management.

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Contents

Chapter 1

Introduction

1.1. Social Environment Surrounding Small Cetaceans

Growing human populations and economic activity affect the marine environment in various ways, including effects from marine development, pollution, overfishing, and the introduction of invasive species. These impacts result in habitat alteration or loss and even the extinction of species. Biodiversity loss is now of great concern globally.

Small cetaceans are important species not only because many are endangered and threatened by anthropogenic disturbances but also because they are top predators in marine ecosystems. Top predators are considered as key species for maintaining the marine ecosystem because the predation risk has considerable effect on the marine trophic cascade and community by inflicting mortality and inducing behavioral modifications on prey and subsequent lower levels of species in a food chain (Heithaus et al., 2008; Verity and Smetacek, 1996). Because of their position in the food chain, cetaceans are useful to monitor ecological integrity.

Recent social demands for the protection of marine diversity have accelerated the introduction of Marine Protected Areas (MPAs). Although there is no conclusive definition of an MPA, the International Union for Conservation of Nature (IUCN) has defined an MPA as “A clearly defined geographical space, recognized, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values” (IUCN 2008). In the

Convention on Biological Diversity (CBD)-tenth meeting of the Conference of the Parties (COP 10), it was declared that “10% of coastal and marine areas, especially areas of particular importance for biodiversity and ecosystem services, are conserved through effectively and equitably managed, ecologically representative and well-connected systems of protected areas and other effective area-based conservation measures” to improve the status of biodiversity by safeguarding ecosystems, species, and genetic diversity. Many studies have suggested that the MPAs can be an effective tool to mitigate anthropogenic impacts on marine mammals (reviewed by Hoyt, 2011). However, this assertion has to take into account the ecology of the target species, such as resting, socializing, feeding, or choice of breeding ground (Ashe et al., 2010; Hoyt, 2011). Both the distribution and the spatiotemporal habitat use data are necessary to establish effective MPAs. A study used behavioral data to establish a conservation strategy for terrestrial animals (Jeffries and Brunton, 2001); however, this kind of approach is not widely used for MPAs. One explicit reason for this is a lack of basic ecological information regarding small cetaceans compared with terrestrial animals.

1.2. Passive Acoustic Monitoring

1.2.1. Development of Acoustic Monitoring

A traditional approach to observing wild populations of small cetaceans is through visual observation from the water surface. Previous studies have revealed the distribution, population density, surface behavior, or habitat use of animals based on visual observations. However, the information accessible from the surface represents merely a fraction of their total behavior manifested when cetaceans rise to the surface and only during daylight hours and during relatively good weather conducive to visual observations. In particular, in the case of hard-to-find species such as the harbor porpoise *Phocoena phocoena* and finless porpoise *Neophocaena asiaeorientalis*, effective visual observations cannot be performed at sea during wind speeds above the Beaufort wind scale 2 (light-breeze) (Evans and Hammond, 2004; Shirakihara et al., 2007). An additional problem is that the quality of information can show high variation between observers or between surveys because of the differences in observation experience or limitation of survey effort among individuals and surveys, respectively.

Within recent decades, acoustic observation has seen widespread use for research on small cetaceans as a complementary method of visual observation (Mellinger et al., 2007). In passive acoustic monitoring (PAM), we record the underwater sounds emitted from animals to obtain ecological data such as behavior, distribution, population density, or habitat usage. Notably, acoustics is almost the only tool that allows long-term observation of submerged animals that are not visible from the surface, and PAM has the added advantage of not interfering with the behavior of animals, and its use is not restricted by the availability of visible light or unfavorable weather conditions.

Small cetacean calls can be roughly classified into two categories based on physical features: tonal sound and pulsed sound (Fig. 1.1). The tonal sound can be described as a "whistle," which has a relatively long duration over a narrow frequency band (2–35 kHz) and generally with frequency modulation. The pulsed sound has a shorter duration over a broad frequency band (5–150 kHz) and can be further categorized into two different types based on the general function: burst pulse and clicks. Burst pulse and whistle calls are considered to be used for intraspecies communication. On the other hand, clicks are mainly used within biosonar signals. It is known that small cetaceans emit clicks and listen to the echoes of sounds bouncing off various objects to achieve environmental cognition underwater. This phenomenon is called echolocation. Many small cetaceans frequently emit clicks at a rate of >1 click min^{-1} on average, e.g., every 12.3 s in the harbor porpoise and every 6.4 s in finless porpoise on average (Akamatsu et al., 2007). We can easily determine whether a small cetacean species is located within the monitoring area by detecting clicks. It has been confirmed that the probability of animal detection using PAM is higher than that by visual observation for some species [e.g., Yangtze finless porpoise *Neophocaena asiaeorientalis asiaeorientalis* (Akamatsu et al., 2008; Kimura et al., 2013), the Ganges River dolphin *Platanista gangetica gangetica*, and the Irrawaddy dolphin *Orcaella brevirostris* (Akamatsu et al., 2013)]. PAM is now introduced at many research sites as the standard monitoring method.

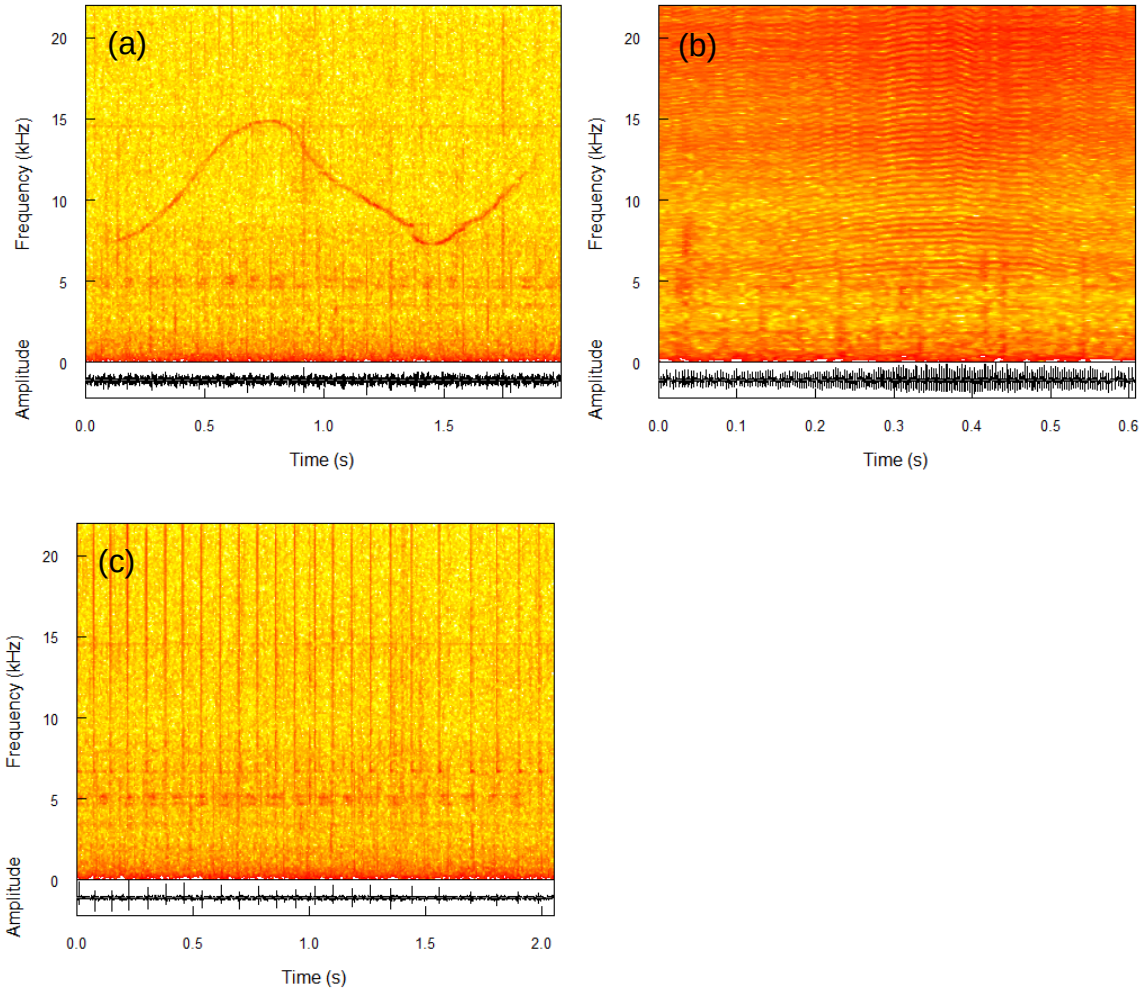

Fig. 1.1 The spectrogram of a whistle (a), burst pulse (b), and click (c) recorded from a bottlenose dolphin [44.1 kHz sampling rate, 512 points of Fast Fourier Transform (FFT) size, hamming window, and 50% overlap].

1.2.2. Acoustic Monitoring Device: A-tag

There are three types of PAM based on the difference of survey platforms. A towed platform of PAM is generally employed simultaneously with visual observations. When using this method, a stand-alone underwater recorder or hydrophone with a cable is towed from the rear of the research vessel. Many studies have used this method to compare the probability of detection of PAM to that using visual observation (Akamatsu et al., 2008) and wide-range spatial distribution (Dong et al., 2011).

When using the stationary type of PAM, a stand-alone autonomous underwater recorder is used within a fixed monitoring point. The device is generally fixed on a

mooring, buoy, or the sea floor and can operate without human intervention after deployment. This method enables continuous long-term monitoring to determine the seasonal change in temporal distribution (Kimura et al., 2012; Verfuß et al., 2007) and population density (Hildebrand et al., 2015; Marques et al., 2013). More than 40 types of different autonomous underwater recorder have been developed (Sousa-Lima et al., 2013).

The bio-logging platform is additionally provided for PAM. Bio-logging is a relatively novel approach compared with the two previously mentioned survey platforms and is defined as “The investigation of phenomena in or around free-ranging organisms that are beyond the boundary of our visibility or experience” (Boyd et al., 2004). In the case of PAM, the stand-alone underwater recorder is attached to the animal. This method is commonly used in conjunction with another device, such as an accelerometer, thermometer, depth meter, or camera to obtain behavioral data from the same individual. The D-tag is a representative device that can record sounds, depth, temperature, and acceleration along with geomagnetic data in the latest version (Johnson and Tyack, 2003; http://soundtags.st-andrews.ac.uk/dtags/).

Although there are an enormous number of available recording devices, most can be used for only one of the three types of survey platforms. The acoustic tag (A-tag; Fig. 1.2) was initially invented for the bio-logging type of PAM (Akamatsu et al., 2005); however, it can also be used within a fixed and towed survey without major mechanical changes. The A-tag (ML200-ASII; Marine Micro Technology, Inc., Saitama, Japan) is an event recorder targeting the biosonar sounds (clicks), which records sound pressure and the time difference of arrival of sound (TDOA) between two hydrophones when the sound is higher than the preset threshold (138 dB peak-to-peak re 1 μPa). The A-tag does not store the waveform of sounds and consists of two hydrophones placed 60 cm apart, a central processing unit (CPU) (PIC18F6620; Microchip, USA), a 128-MB flash memory, and 2 UM1 batteries. The CPU, flash memory, and batteries are housed in a waterproof aluminum case (Fig. 1.2). A passive two-pole bandpass filter of 55–235 kHz, which includes the peak frequency of major small cetacean species such as harbor porpoise (129–145 kHz; Villadsgaard et al., 2007) and bottlenose dolphin (23–134 kHz; Au, 1993), was used.

The sensitive frequency of each hydrophone can be chosen at approximately 130 kHz or 70 kHz according to the target species.

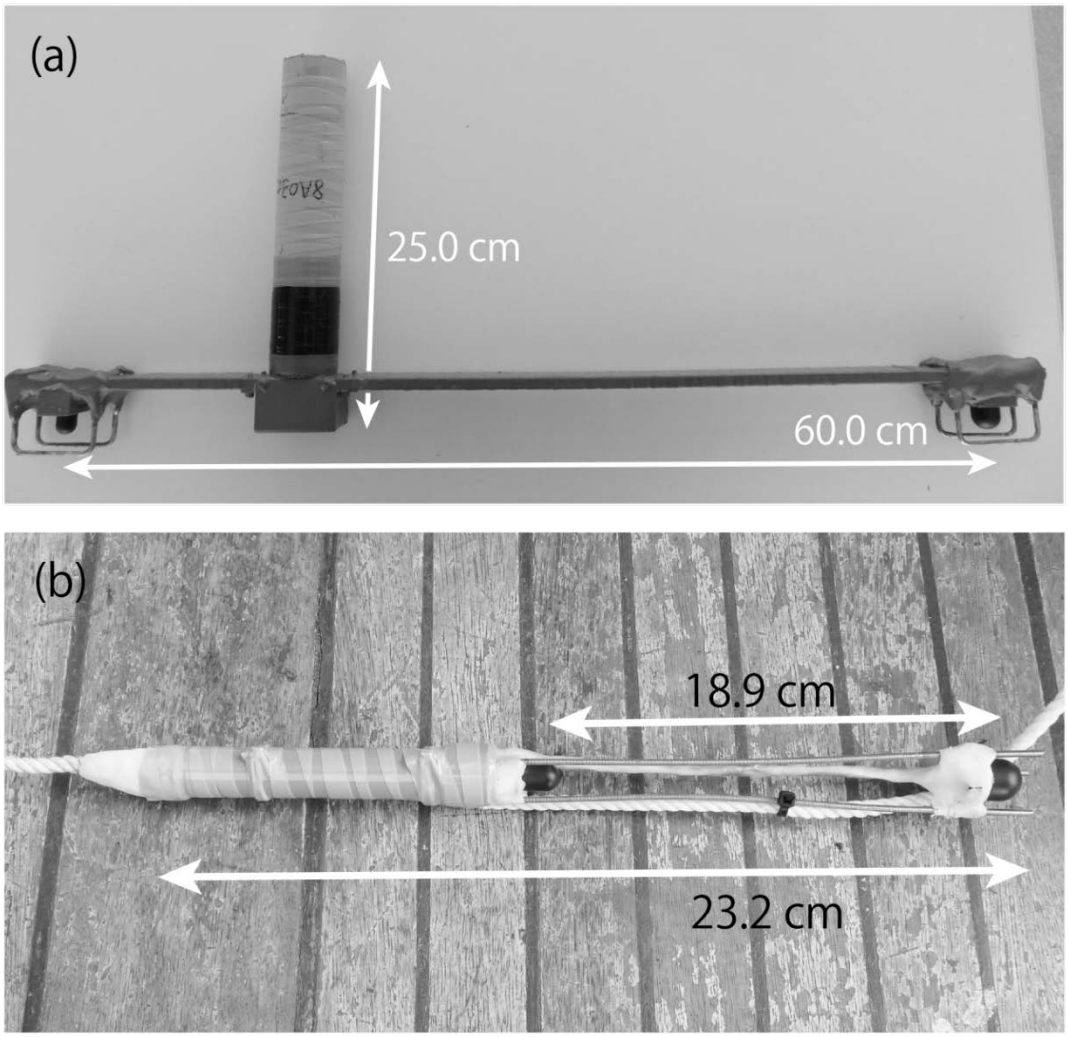

Fig. 1.2 The fixed type (a) and towed type (b) of A-tag.

1.3. Study Area

1.3.1. The Istanbul Strait (Bosphorus)

The Istanbul Strait is located as the boundary between Europe and Asia which connects the Black Sea and the Marmara Sea (Fig. 1.3). It is one of the narrowest channels in the world with approximately 31 km long, 0.7–3.5 km wide and 35.8–110 m deep (Alpar and Yüce, 1998). The strait is consisted 2 layer of water column because of the difference of water density, caused by different salinity and temperature, between the Black Sea and the Mediterranean Sea. Moreover, the water flow of each layer is opposite. The upper layer, which is consisted of the low salinity water from the Black Sea (approx. <20), flows from the Black Sea toward the Marmara Sea. On the other hand, the lower layer which is consisted of the high salinity water from the Mediterranean Sea (approx. >30), flows from the Marmara Sea toward the Black Sea (Tarkan et al., 2005; Yüce, 1996). This specific oceanographic feature functions as the biological barrier or corridor for Mediterranean or Black Sea species. The high salinity of the Black Sea water likely limits the distribution of some marine creatures. For example, 11 cephalopod species has been recorded in the Marmara Sea but no cephalopod is known from the Istanbul Strait and the Black Sea. The recorded decapod species is 177 in all Turkish water but only the 17 species can be found in the Black Sea (Öztürk and Öztürk, 1996). The benthic species is poor in the Black Sea water when compared with the Mediterranean Sea and the Marmara Sea because of the low salinity and the an intensive anoxic water.

Since the strait is the only water way connecting the Black Sea to Mediterranean Sea through the Marmara Sea, it is important corridor especially for the economies of the countries around the Black Sea. Official government statistics reported that 130 commercial cargo vessels and 2500 domestic vessels pass through the strait per day on average (Baş et al., 2015).

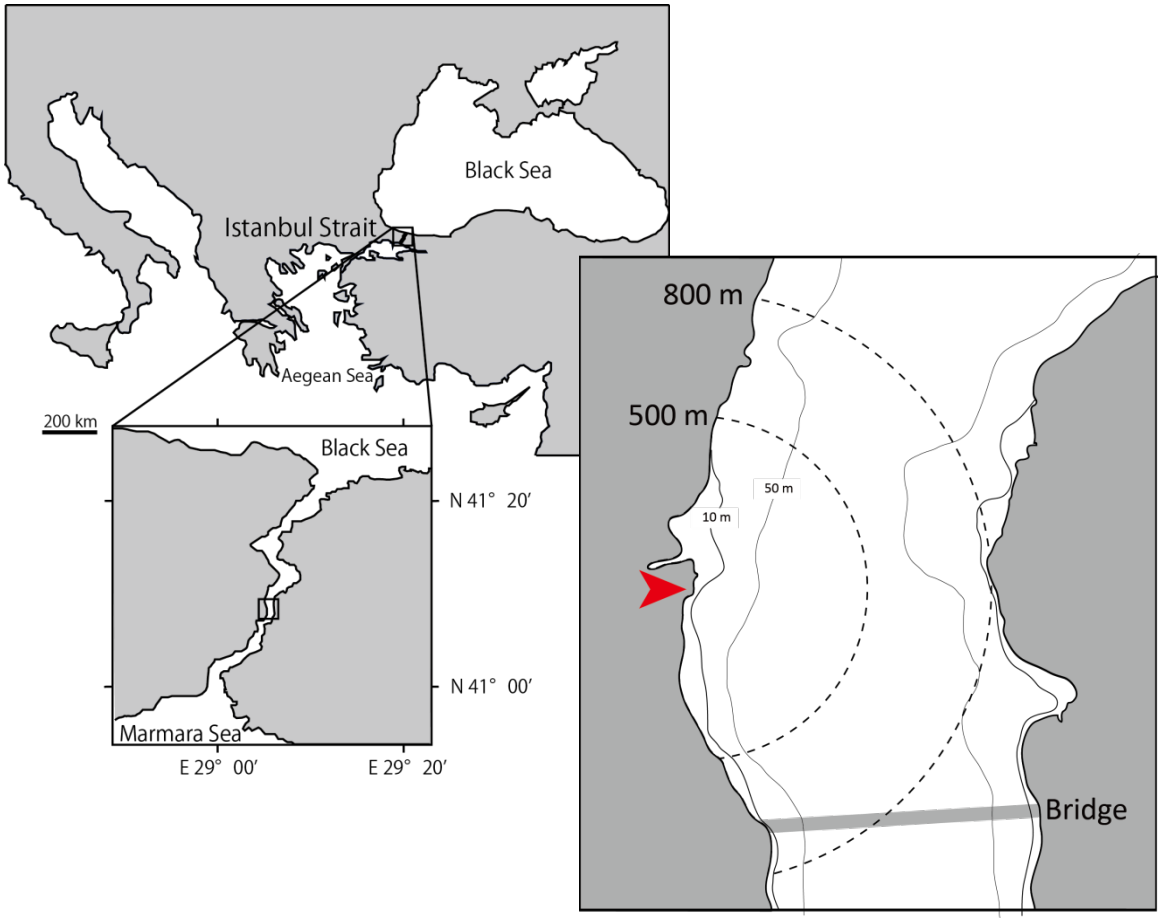

Fig. 1.3 Study area, the Istanbul Strait. The red arrow indicates the fixed acoustic observation point.

1.3.2. Small Cetaceans

Three small cetacean species from two families are regularly observed in the strait: one porpoise species, namely the harbor porpoise *P. phocoena* and two delphinids, namely the bottlenose dolphin *Tursiops truncatus* and the short-beaked common dolphin *Delphinus delphis* (Fig. 1.4–1.6). All three species are considered as genetically and morphologically independent subspecies that mainly inhabit the Black Sea. The species we observed in the strait possibly belong to these subspecies. Only the harbor porpoise was not only genetically and morphologically but also geographically isolated from the other populations approximately 5,000 years ago (Fontaine et al., 2010). Although observed delphinids in the strait were considered as belonging to the Black Sea subspecies, the extent of the area used as a habitat range within the region bounded by the Black Sea to the north eastern Aegean Sea through the Marmara Sea and Canakkale Strait (Dardanelles) remains unknown. All the observed delphinids are considered as endangered subspecies; the harbor porpoise and delphinids are listed as "Endangered," whereas the short-beaked common dolphin is listed as "Vulnerable" by IUCN Red List of Threatened Species (Birkun 2008, 2012; Birkun et al., 2008). The Istanbul Strait, the Marmara Sea, and the Canakkale Strait are reported as important areas for all small cetacean species within the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea, and contiguous Atlantic Area (ACCOBAMS), which was signed among 23 countries in Europe (Notarbartolo and Birkun, 2010; http://www.accobams.org/index.php). These areas are proposed as MPAs for the Black Sea small cetaceans; however, Turkey has to date not signed the agreement, and no MPAs currently exist along the Turkish coast (Notarbartolo di Sciara and Birkun, 2010; Baş et al., 2014).

Early findings suggest that short-beaked common dolphin migrate from the Mediterranean Sea to the Black Sea through the Marmara Sea and the Istanbul Strait during spring and return during autumn (Öztürk and Öztürk, 1996). In addition, a group of bottlenose dolphins and short-beaked common dolphins were observed to migrate from the Aegean Sea toward the Marmara Sea during April–May (Berkes, 1977). The trigger for these migrations is considered to be pelagic fish, such as sand smelt *Atherina boyeri*, sprat *Sprattus sprattus*, anchovy *Engraulis encrasicolus* and horse mackerel *Trachurus trachurus*, which migrate from the Aegean Sea or the Marmara Sea to the Black Sea during spring (Berkes, 1977; Dede et al., 2013; Öztürk and Öztürk, 1996).

However, regular migration might be prevented by the current heavy marine traffic and other ecological stresses affecting the strait (Öztürk and Öztürk, 1996). In addition, detailed information of the timing of migration, local distribution, behavior of each small cetacean species, or explicit evidence of migration in recent years is lacking.

Fig. 1.4 Bottlenose dolphins (Photo by Ayhan Dede).

Fig. 1.5 Short-beaked common dolphins (Adopted from Protected Resources Division, Southwest Fisheries Science Center, La Jolla, California. swfsc.nmfs.noaa.gov/PRD/)

Fig. 1. 6 Black Sea harbor porpoise ((a) Photo by Sergey Krivokhizhin /Brema Lab. Adopted from Notarbartolo di Sciara and Birkun 2010; (b) Photo by Arda M. Tonay)

1.4. Objectives

The objectives of the current study are to develop the PAM methodology for multiple species observation and to obtain ecological information for small cetaceans observed in the Istanbul Strait. As mentioned in section 1.2, PAM is now widely used for small cetacean research; however, the simultaneous observation of multiple species is challenging because of the methodological limitations of discriminating between multiple species by acoustics. Because the ecology of each species must be different, especially in harbor porpoise, the ecology of each species must be determined for effective conservation, including seasonal behavior changes, animal density, and distribution.

1.5. Structure of Thesis

The outline of this thesis is organized as follows:

- Chapter 2: describes a method for discriminating between species using acoustics, between the harbor porpoise and delphinids.
- Chanter 3: describes a proposed new acoustic-based density estimation approach for application over a wide area with consideration of species discrimination accuracy.
- Chapter 4: describes the long-term seasonal change of occurrence and acoustic behavior in each family after adapting the newly developed species discrimination method described in Chapter 2
- Chapter 5: describes the wide-range distribution and estimated animal density after adapting the newly developed species discrimination method described in Chapter 2 and the proposed density estimation approach described in Chapter 3.
- Chapter 6: is general discussion regarding the effectiveness and limitations of the newly developed PAM methodology in Chapter 2 and Chapter 3. In addition, this chapter integrates the results of Chapter 4 and Chapter 5 with implications for the conservation of small cetaceans in the Istanbul Strait.

Chapter 2

Acoustic Family Discrimination Method

2.1. Introduction

PAM has been widely used in cetacean research (reviewed by Mellinger et al., 2007), especially to determine the presence and abundance of species by using towed hydrophone arrays (e.g., Barlow and Taylor, 2005) and the long-term trend or habitat by using fixed hydrophone systems (Kimura et al., 2010; Verfuß et al., 2007). In recent years, such monitoring systems are used not only for detecting the presence of animals but also in identifying the phonating species. For example, baleen whale calls are appropriate targets to identify the location and species because of their stereotype structure. Combination of low-frequency pulsed calls, or A calls, and low-frequency tonal calls, or B calls, of blue whales in the Pacific Ocean can be easily discriminated from ambient noise in offshore calm waters (Oleson et al., 2007). These call features also show geographic differences between populations in the Atlantic Ocean and those in the Pacific Ocean (Gavrilov et al., 2012; Mcdonald et al., 2006). Only male humpback whales and fin whales generate sequences of calls, which are considered useful for mate selection during their breeding season (Croll et al., 2002; Darling and Berube, 2001; Payne and McVay, 1971). The sequence of specific call types of these whales can be differentiated from other sound sources.

In the case of dolphins, whistles are considered as acoustic keys for species identification. A previous study showed that the female bottlenose dolphin calls showed

the same contour on a spectrogram for about 12 years (Quick and Janik, 2012; Sayigh et al., 1990). Oswald et al., (2007) developed a Real-time Odontocete Call Classification Algorithm (ROCCA) for nine species of dolphins, based on the basic acoustic features in whistles. However, vocal learning in small cetaceans, as shown in killer whales (Deecke et al., 2000) and bottlenose dolphins (Quick and Janik, 2012), could cause changes in vocal characteristics, thus, making it further difficult to identify the species.

On the other hand, the spectrum and waveform of biosonar sounds in porpoises and delphinids show clear differences and are relatively stable within the same individual and the same species. Most of delphinids use broad-band signals. Its biosonar sound contains energy that encompasses a wide range of frequencies, ranging from 23 kHz to 134 kHz (Au, 1993), and its power spectrum level shows a relatively flat shape. In contrast, the porpoises, including the harbor porpoise, uses narrow-band high-frequency signals with peak energies of approximately 130 kHz (Madsen et al., 2005; Villadsgaard et al., 2007). Soldevilla et al., (2008) suggested that even among delphinids, classifying Risso's dolphin and the Pacific white-sided dolphin is possible based on clicks.

The timing porpoise detector (T-POD) and its successor, C-POD, were developed to differentiate the harbor porpoise from the delphinids by using these acoustic characteristics of clicks. This equipment has been extensively used in field studies (Gallus et al., 2012; Philpott et al., 2007; Todd et al., 2009; Verfuß et al., 2007). T-POD and C-POD are efficient systems for long-term fixed PAM. However, they have device-dependent algorithms and different versions of T-POD or C-POD show variable detection performances (Kyhn et al., 2008; Verfuß et al., 2013).

In recent years, many types of PAM devices have been developed and installed in the ocean to monitor cetaceans and other marine animals (Sousa-Lima et al., 2013). For quantitative comparisons among these observations, simple acoustic characteristics are needed for species or family identification. Ideally, the method should not depend on device type and should discriminate species with degree of precision.

In this chapter, we developed the simple discrimination method between harbor porpoise and delphinids by using the two-band frequency intensity comparison (130 kHz and 70 kHz) in the Istanbul Strait in Turkey (Kameyama et al., 2014). In this

method, the correct detection and false alarm rates of discrimination can be estimated. In addition, I propose a model that can quantify how the proposed method works under various mix ratios of sounds from each family.

2.2. Materials and Methods

2.2.1. Acoustic Observation

The A-tag (ML200-ASII; Marine Micro Technology, Inc., Saitama, Japan) was deployed in the middle of the Istanbul Strait (Fig. 1.3; 41.05' 835" N, 29.03' 264" E) between April 12 and June 1, 2012, when the three species were expected to appear in the strait (Öztürk and Öztürk, 1996). An iron pipe and a basket that were fixed beside the pier located at the bank of the Istanbul Strait were used to set the A-tag approximately 35 cm deep from the bottom. The tide level change was within 34 cm in this area (Alpar and Yüce, 1998). This is smaller compared to the depth of the A-tag, which was not exposed to air at any time. The depth at the deployed point was 125 + 34 cm depending on the tide level (Fig. 2.1).

The maximum detection range of the A-tag was calculated using the following parameter: maximum source sound pressure level (Source SPL), which was estimated at 227 dB peak-to-peak re 1 µPa for bottlenose dolphins (Au, 1993; Simard et al., 2010) and 205 dB peak-to-peak re 1 µPa for harbor porpoise (Villadsgaard et al., 2007); the minimum received sound pressure level of the present A-tag (Received SPL: 138 dB peak-to-peak re 1 µPa); and the absorption coefficient of the Istanbul Strait (α). Assuming average salinity of upper layer was 17.5, temperature was 15 degrees Celsius, α was estimated at 0.026 dB m^{-1} for 130 kHz and 0.013 dB m^{-1} for 70 kHz by using the Francois and Garrison model (Francois and Garrison 1982a; Francois and Garrison 1982b). The maximum detection range d was estimated using Eq. (2.1).

$$\text{Source SPL} = \text{Received SPL} + 20\log_{10}(d) + \alpha d \qquad (2.1)$$

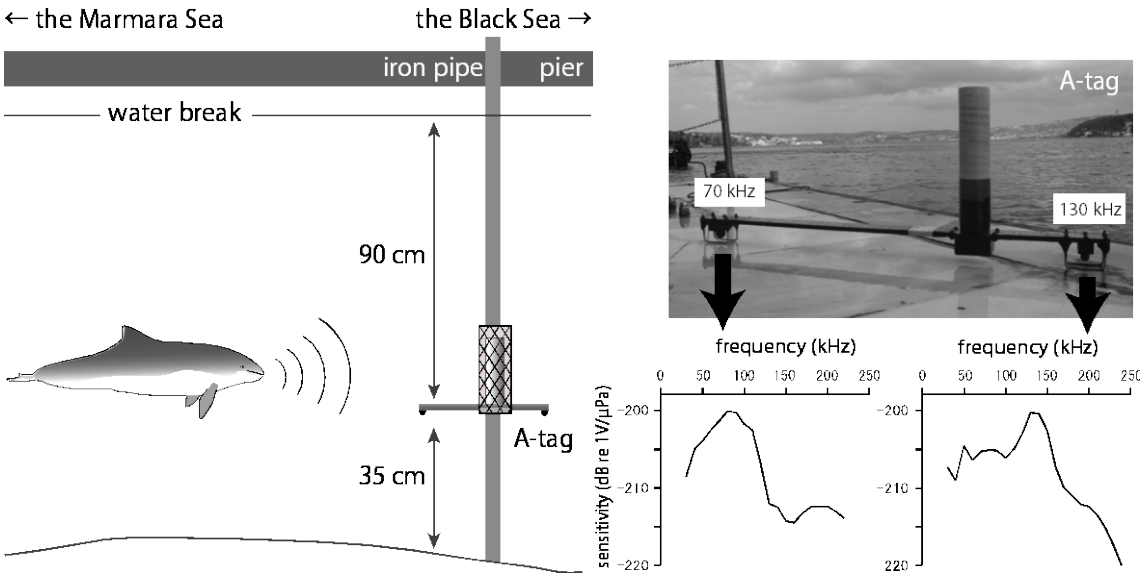

Fig. 2.1 Left: The A-tag was fixed using an iron pipe and a basket. Right: A photo of the A-tag (upper) and the frequency responses of each hydrophone (lower). Each hydrophone has different frequency sensitivity: The primary hydrophone is the most sensitive at 70 kHz and the secondary hydrophone is the most sensitive at 130 kHz.

2.2.2. Visual Observation

Visual observations were conducted simultaneously with the acoustic observations. During visual observations, the time, species, distance, angle from the observation site, group size, and swimming direction were recorded on each sighting. The time and species were used for analysis in this study. Two observers conducted observations from the bank side of a pier where the A-tag was deployed. Two to four persons participated in the visual observations. Two persons were on-duty, covering 90° each, thus, ensuring the monitoring of a 180° sector from the observation point to the opposite shore. One observation interval lasted 30 min, and the position was changed alternately. If more than two observers were available, the remaining observers rested during off-duty periods.

2.2.3. Off-Line Data Analysis

I applied an off-line filter using Igor Pro 6.2.2 (WaveMetrics, Inc., Oregon, USA) for data collection to exclude false positives that were generated by background noise. Firstly, I excluded the data that were not triggered by both hydrophones before applying the filtering. The off-line filter consisted of three steps. In the first step, the surface or bottom reflections, which could be recorded within 2.5 ms at most from the direct path sound, were excluded. We defined the boundary of different click trains at 200 ms pulse intervals (Akamatsu et al., 2007). If the click sequences were separated by more than 200 ms, each sequence was considered a different click train. In the second step, the minimum number of clicks in a click train was defined. If there were only a few clicks in a click train, it was highly likely that these were background noise. I extracted the click trains that contained six or more pulses in the train (Akamatsu et al., 2010a; Kimura et al., 2010). In the third step, the click trains that showed irregular changes in inter-click intervals were eliminated. The inter-click intervals of biosonar sounds in delphinids and porpoises should gradually change (Akamatsu et al., 1998; Li et al., 2009). If the coefficient of variation of the inter-click interval in a click train was below 0.3, these click trains may contain more than two animals' sonar signals or background noise that might have randomly changed the inter-click interval (Kimura et al., 2010). Finally, manual checks were conducted to exclude false positives generated by noise caused by large ships approaching the device or the occasional contamination of artificial sonar sounds. These noises did not occur frequently but masked the data for several 10 s segments. The period of noise contamination was excluded from further analyses.

The ratio of received sound pressure by the two hydrophones (130 kHz divided by 70 kHz) was calculated for each click in a click train. A two-band ratio was defined as the averaged ratios of all clicks in a click train.

Acoustic datasets that clearly matched the visual observation results were selected for analysis. If I sighted one of the three species—harbor porpoise, short-beaked common dolphin, or bottlenose dolphin—I considered that the click trains recorded ± 10 min from the sighting were generated by the sighted animals. I excluded the click trains that were recorded when both harbor porpoise and delphinids were

sighted within ± 10 min periods. The click trains without visual confirmation were not used, because it was difficult to determine the species that produced the click trains. Finally, I obtained the two-band ratio of each click train with species confirmation.

2.2.4. Species Discrimination Model

Histograms of the two-band ratios were depicted for the two families. These distributions could be overlapped because some of the two-band ratios of harbor porpoises could be the same or even smaller than those of delphinids and vice versa. A simple definition of an appropriate threshold of two-band ratios to discriminate species is the intersection point of two distributions (see Fig. 2.2a observed distribution). Hereinafter, I refer the intersection point of two distributions of observed data as the fixed threshold. To determine the intersection point, I used a 0.1 bin histogram. This simple method to discriminate species also includes some false alarm (indicated as FD and FP in Fig. 2.2a). The shape of the two-band ratio distribution is assumed to be the same for each species regardless of the data size if enough data was used to develop the distribution. However, the intersection point of each distribution clearly changes depending on the mix ratio of detected number of click trains in each species. For example, if the portion of click trains recorded from delphinids is higher than that from porpoises, the fixed threshold should shift toward harbor porpoise side. To adjust this kind of changes of mix proportions, I propose a method to calculate the dynamic threshold level of the two-band ratio.

Correct detection and false alarm are the functions of an arbitrary threshold (x). Ideally, the summation of the number of correct detections of delphinids (TD) and that of harbor porpoise (TP) should be maximized. Similarly, the number of false alarms of delphinids (FD) and that of harbor porpoise (FP) should be minimized. Thus, I define the value of the index of classification efficiency, Index(x), as shown in Eq. (2.2):

$$\text{Index}\ (x) = \frac{TD(x) + TP(x)}{FP(x) + FD(x)} \tag{2.2}$$

To identify the maximum value of index of classification efficiency according to threshold (x), the differential of the index of classification efficiency by threshold (x) should be zero, as shown in the following Eq. (2.3).

$$\frac{\Delta \text{ Index } (x)}{\Delta \text{ threshold } (x)} = 0 \tag{2.3}$$

The dynamic threshold is when threshold (x) satisfies Eq. (2.3). In reality, the function of TD(x), TP(x), FD(x), and FP(x) are given numerically based on the acoustic identification compared to the visual ground truth data. Therefore, the index of classification efficiency was also calculated numerically.

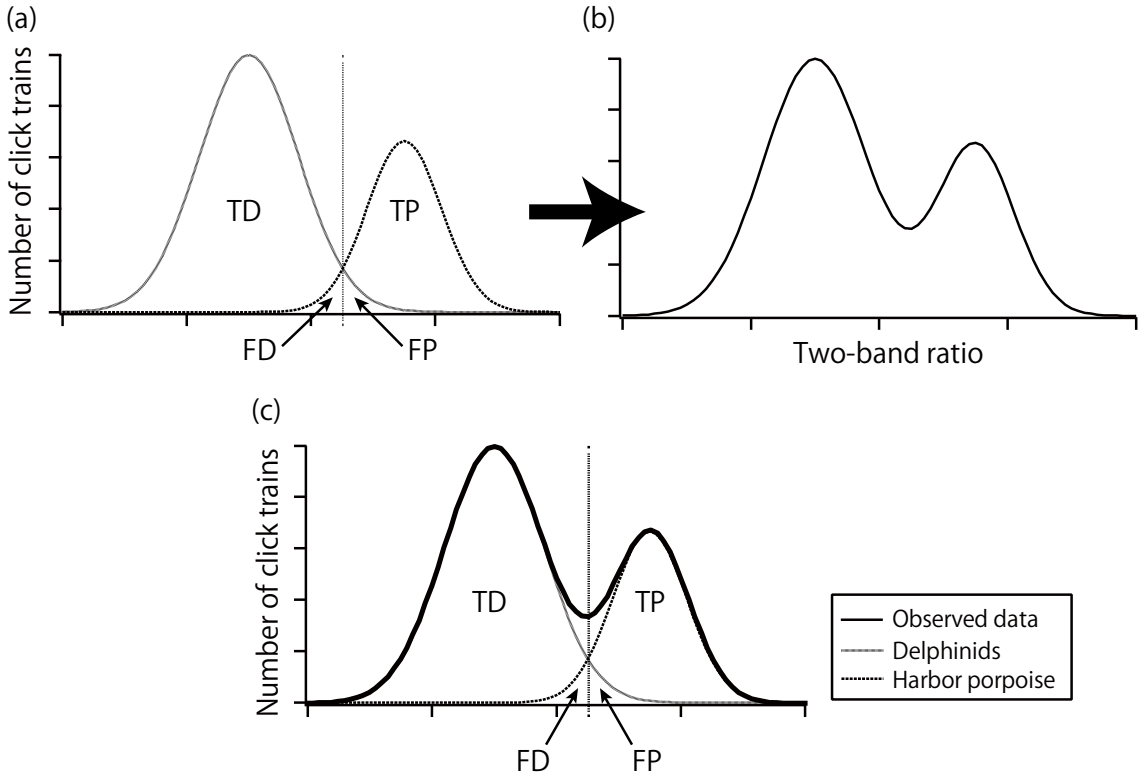

Fig. 2.2 The diagram of the two-band ratio with the correct detection of delphinids (TD) and the harbor porpoise (TP) and the false alarm for delphinids (FD) and the harbor porpoise (FP) and how to estimate the appropriate dynamic threshold from the observed acoustic data based on grand truth distribution. TD and FD are the number of click trains up to the threshold, and FP and TP are the rest of them. (a) The dynamic threshold with the correct detection rate and false alarm rate in each mix ratio were calculated in this study by using grand truth distribution. (b) Bimodal distribution of the delphinids and harbor porpoise were generated from acoustic observations. The mix ratio of each family is unknown in these data. (c) To estimate the appropriate mix ratio of (b), (a) can be used as the validation function by minimizing the differential of (a) and (b).

2.2.5. Model Validation

The proposed models of fixed and dynamic thresholds were validated using observed data with changing proportions of click trains in each species. Mix distributions of the two-band ratio, which contains the variable ratio of each family from 0% to 100%, were created. The portions of click trains of harbor porpoise and delphinids were β and (100-β), where $0<\beta<100$. I calculated the dynamic threshold that maximizes the index of classification efficiency [see Eq. (2.2)-(2.3)] in each mix ratio. The fixed and dynamic thresholds were provided as a function of the mix ratio between numbers of click trains, β. Applying fixed and dynamic thresholds to the calculated mix distribution, I could evaluate the correct detection and false alarm rates of each model as follows [Eq. (2.4)-(2.5)]:

$$\text{Correct detection rate} = \frac{TP}{(TP + FD)} \tag{2.4}$$

$$\text{False alarm rate} = \frac{FP}{(TD + FP)} \tag{2.5}$$

(2.4), (2.5) is for harbor porpoise. *P* and *D* should be inverted for delphinids.

For quantitative evaluation, Matthews correlation coefficient (MCC) was introduced (Baldi et al., 2000; Matthews, 1975), as shown in Eq. (2.6). MCC was used in machine learning to evaluate the quality of binary classifications that can be considered both correct detection and false alarm rate using one value. When FD and FP are zero, the value becomes 1, which indicates perfect classification. On the other hand, when either TD or TP is zero, the denominator becomes zero, which indicates that the classification failed.

$$\text{MCC} = \frac{(TP \times TD) - (FP \times FD)}{\sqrt{(TP + FP)(TP + FD)(TD + FP)(TD + FD)}} \tag{2.6}$$

Once the threshold was derived, it was possible to estimate the mix ratio β of two families and the appropriate threshold. The acoustic data can only provide a bimodal distribution of the two-band ratio of both harbor porpoise and delphinids (Fig. 2.2). Assuming that the distribution of the two-band ratio of each family is nearly stable, the distribution obtained in the present study can be used as ground truth (Fig. 2.2). A difference in the observed distribution of the two-band ratio and a linear combination of ground truths of the two families were used as a validation function to estimate the appropriate threshold, as shown in Eq. (2.7). The image of threshold estimation is shown in Fig. 2.2c.

$$\text{Difference} = \int \left|\text{observed} - \left((100 - \beta)\text{delphinids} + \beta * \text{harbor porpoise}\right)\right| \quad (2.7)$$

The "difference" is the difference between observed data, which is the data of sound pressure ratio observed in other experiments, and the grand truth data, which I used to calculate the threshold in this study. This "difference" should be minimized to estimate the closest model (i.e., appropriate β) to the observed data. The "observed," "delphinids," and "harbor porpoise" represent the number of click trains in each β that is the mix coefficient of each family's distribution.

2.3. Results

The recordings of 639 click trains of delphinids and 104 click trains of harbor porpoises with visual species confirmation were obtained after off-line filtering. The distribution of the two-band ratios is presented in Fig. 2.3. The fixed threshold (0.90) is represented by an arrow in Fig. 2.3. The index of classification efficiency was depicted according to Eq. (2.2) and the arbitrary threshold (x) in Fig. 2.4. The graph has a simple one-peak shape, and the dynamic threshold was defined as 0.82 that maximizes the index of classification efficiency. The dynamic threshold was slightly lower than the fixed threshold level of 0.90. The receiver operating characteristics (ROC) curve was used to describe and compare the performance of binary classification (Fig. 2.5). I changed the threshold in 0.01 steps from 0 to 3 and calculated the correct detection rate

and false alarm rate. When the fixed threshold of 0.90 was used, the harbor porpoise showed a correct detection and false alarm rate of 0.55 and 0.06, respectively. When the dynamic threshold of 0.82 was used, the harbor porpoise showed a correct detection and false alarm rate of 0.74 and 0.08, respectively. On the other hand, delphinids showed a correct detection and false alarm rate of 0.93 and 0.45, respectively, when the fixed threshold was used and 0.92 and 0.26, respectively, when the dynamic threshold was used. The present mixed families data correspond to $\beta = 14$, suggesting that the detection of click trains from delphinids were dominant. In this situation, false alarm rate of delphinids was large due to incorrect categorization of harbor porpoise sounds as those of the delphinids.

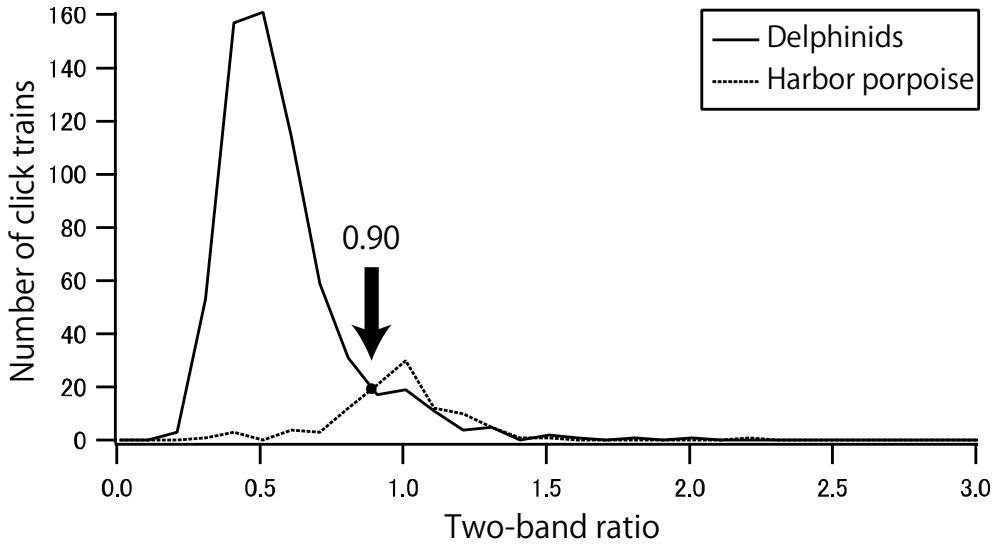

Fig. 2.3 Distribution of the two-band ratio of the observed click trains. The solid line represents the delphinids and the dotted line depicts the harbor porpoise. An arrow indicates the fixed threshold, and its value was 0.90.

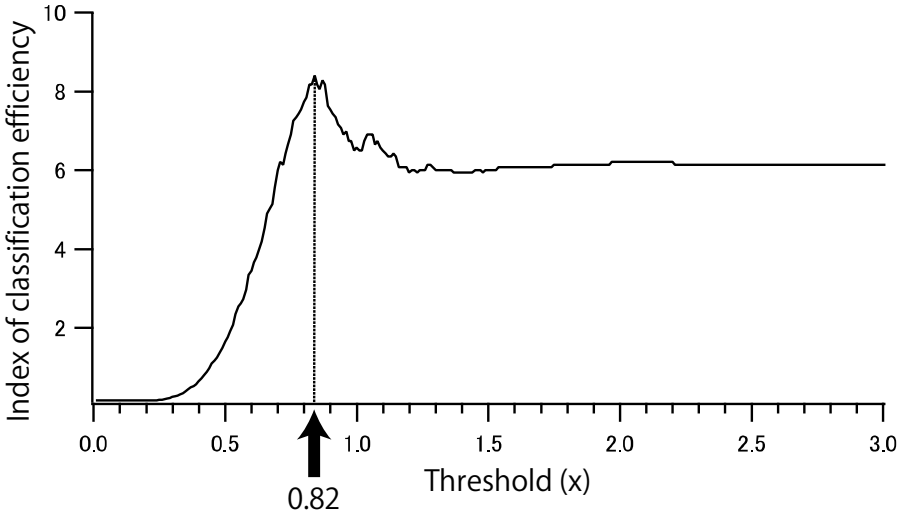

Fig. 2.4 Distribution of the index of classification efficiency calculated from each threshold (x) of the observed two-band ratio as shown in Fig. 2.3. The determined dynamic threshold is indicated by a black arrow (0.82).

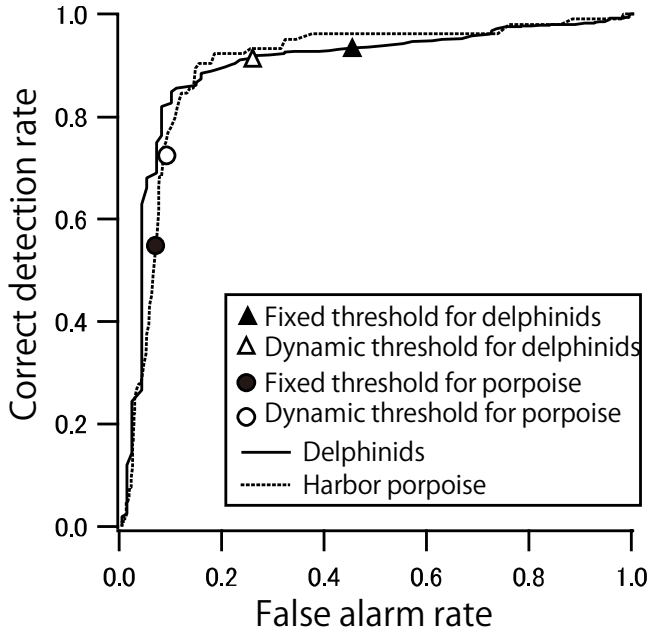

Fig. 2.5 The ROC curve of the observed two-band ratio. The solid line represents the delphinids and the dotted line depicts the harbor porpoise. Black markers indicate the correct detection rate and false alarm rate using the fixed threshold, whereas the white markers indicate those of the dynamic threshold. Triangles indicate delphinids and circles indicate the harbor porpoise, as calculated using the fixed threshold and the dynamic threshold, respectively.

I changed the mix ratio β by conducting a numerical experiment. The calculated dynamic threshold is shown in Fig. 2.6 as a solid line. The dynamic threshold changed from 0.24 to 3.00 depending on the ratio between the number of click trains of harbor porpoises and delphinids β.

The correct detection and false alarm rates proposed by the dynamic threshold and fixed threshold for the created distribution are shown in Fig. 2.7. In the fixed threshold, the correct detection and false alarm rates have a stable value. The correct detection rate was 0.93 for the delphinids and 0.55 for the harbor porpoise. On the other hand, the correct detection rate changed from 0.02 to 1 for the delphinids and from 0.01 to 1 for the harbor porpoise when the dynamic threshold was used (Fig. 2.6). Similarly, the false alarm rate calculated using the fixed threshold was 0.45 for delphinids and 0.07 for harbor porpoise. The false alarm rate calculated using the dynamic threshold changed from 0 to 0.99 for the delphinids and from 0 to 0.98 for the harbor porpoise. Although the correct detection rate was high, the false alarm rate was also high when β was below 10% in the delphinids, and the opposite for the harbor porpoise. In addition, both correct detection rate and false alarm rate were low when β was >90% in the delphinids and the opposite for the harbor porpoise. Between 26% and 82% of the β, the dynamic threshold could identify both the delphinids and the harbor porpoise with over 0.80 of correct detection rate and below 0.20 of the false alarm rate.

The comparison of MCC between the dynamic and fixed threshold is shown in Fig. 2.8. The MCC that was calculated using the dynamic threshold was higher than that calculated using the fixed threshold between 10% and 95% of β.

The maximum detection range of the harbor porpoise was 630 m and that of bottlenose dolphin was more than 1,000 m, which was calculated using the same method as that used for the harbor porpoise.

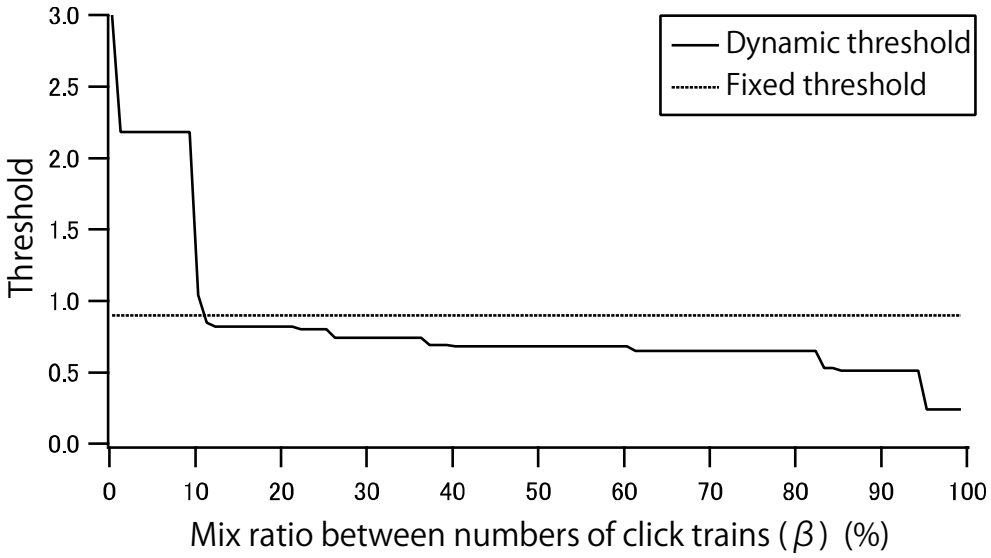

Fig. 2.6 The dynamic threshold (solid line) and fixed threshold (dotted line) in each mix ratio between numbers of click trains, which can also be called mix ratio β. The dynamic thresholds change depending on the mix ratio.

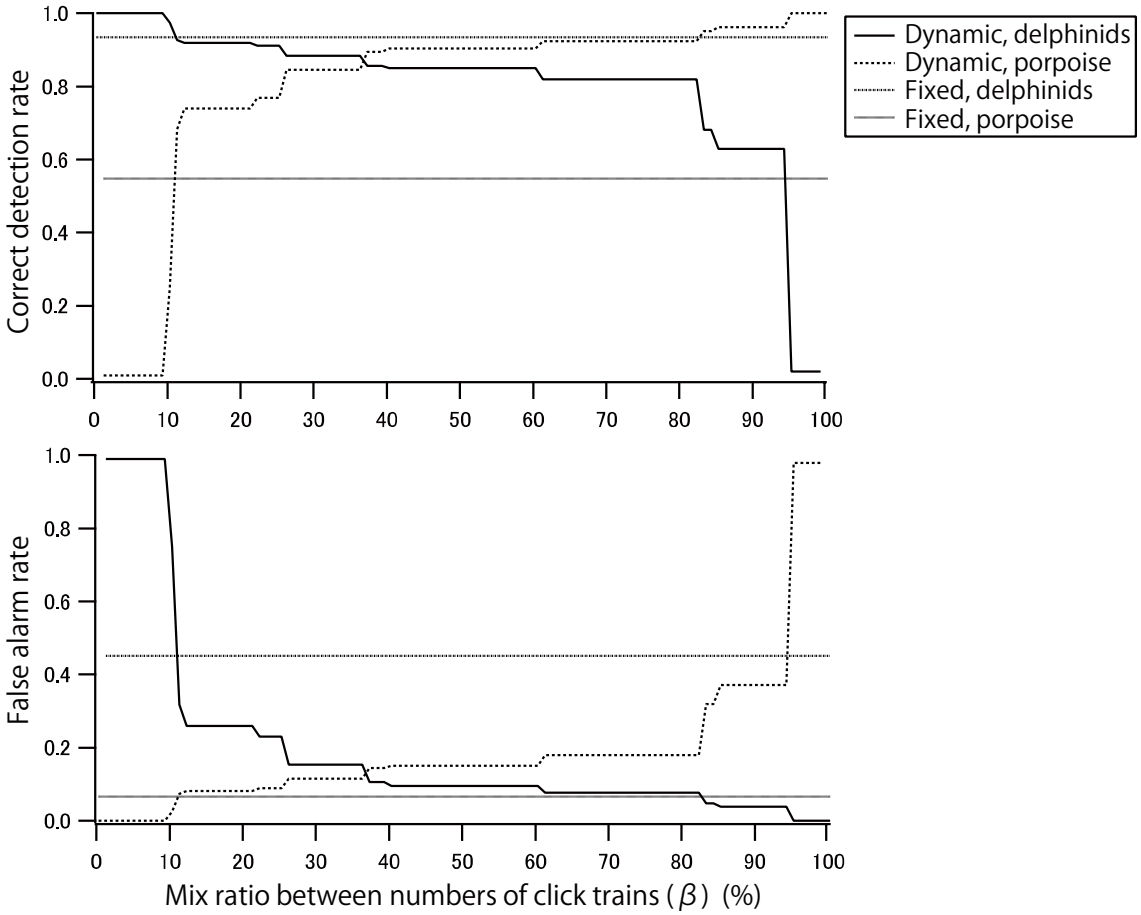

Fig. 2.7 The correct detection rate (upper graph) and false alarm rate (lower graph) of the delphinids and harbor porpoise as provided by the dynamic threshold and the fixed threshold in each mix ratio between the numbers of click trains, which can also be called mix ratio β.

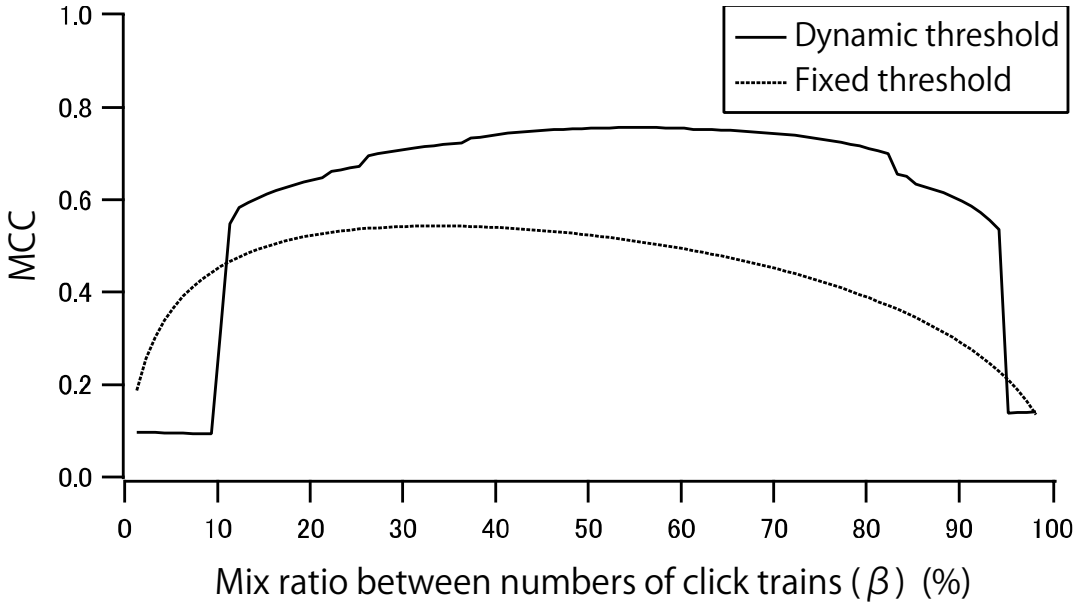

Fig. 2.8 The MCC value provided by the dynamic threshold (solid line) and the fixed threshold (dotted line) in each mix ratio between the numbers of click trains, which can also be called mix ratio β. The value of MCC of the dynamic threshold is higher than that of the fixed threshold, except when the mix ratio between numbers of click trains is below 10% or above 95%.

2.4. Discussion

In this study, I proposed the simplest quantitative species identification method by using the two-band ratio (130 kHz / 70 kHz). As shown in previous studies (acoustic characteristics of biosonar sounds listed in Au, 1993; Wartzok and Ketten, 1999), delphinids use broad-band signals of similar order intensities at both 70 kHz and 130 kHz. On the other hand, harbor porpoises use narrow-band high-frequency signals that have peak intensity at around 130 kHz. These general characteristics are similar no matter where these species live, therefore, the comparisons between two-band ratios would be robust and can be performed using any calibrated hydrophone that is capable of differentiating the two frequencies. Even a single hydrophone with two different band-pass filters could be utilized for this purpose.

However, we have to take into consideration that delphinids also produce a variety of clicks of different spectrum shapes such as on- and off-axis and occasionally

produce narrow-band high-frequency signals during extreme cases (Simon et al., 2010). This is why the statistical evaluation of discrimination performance is needed, as described in this paper. Perfect discrimination of delphinids and the harbor porpoise is impossible because the distribution of the two-band ratio of delphinids partly overlapped with that of the harbor porpoise (Fig. 2.3). Quantitative discrimination performance should be described with detection probability.

I examined the detection performance of both the fixed and the dynamic thresholds, which depend on the mix ratio of recorded sounds from both families. The fixed threshold can be calculated as the simplest constant value of the ratio of two families from the observed data. However, the proposed dynamic threshold showed a greater performance in discriminating families than that by using the fixed threshold (Fig. 2.5 –2.8).

The classification performance was affected by the mix ratio of the detected number of click trains in each family in this study. The performance to discriminate each family was examined by using the ROC curve (Fig. 2.5). The ROC curve of family identification showed that the false alarm rate was lower and the correct detection rate was higher in the upper left area of the graph. Therefore, the upper left area can be considered as the optimal threshold. The result showed that the dynamic threshold (white markers) could identify the harbor porpoise and delphinids better than that by using the fixed threshold (black markers) even under the same mix ratio, which was visually confirmed as the ground truth distribution of two-band ratio in the present study. The white markers were closer together than the black markers in the upper left area in the ROC curves of the harbor porpoise and delphinids (Fig. 2.5). This ROC curve was not available without visual confirmation of the species. Determining the discrimination criteria without ground truth data was the arbitrary choice and was not quantitative in most cases.

By definition, the dynamic threshold changes according to the mix ratio (Fig. 2.8). The difference between the fixed threshold and the dynamic threshold was large when the mix ratio was strongly biased. This is the compensation to minimize false alarm by the dynamic threshold. Large numbers of false alarms could be detected using the fixed threshold because the left or right tail of the distribution could be included (Fig.

2.3). The dynamic threshold did not change smoothly due to the limited sample size of the ground truth data, especially in the harbor porpoise (Fig. 2.3).

The MCC value, which can take into account both correct detection and false alarm rates as one parameter to evaluate classification efficiency, of the dynamic threshold was higher than that of the fixed threshold (Fig. 2.8). This indicates that the dynamic threshold could distinguish each family more accurately than the fixed threshold for various mix ratios of the two families. The fixed or dynamic threshold is not applicable for extremely biased mix ratio of families (Fig. 2.7 and 2.8). The dynamic threshold could not be used when the mix ratio between numbers of click trains is over 95% or under 10%.

The maximum detection ranges were consistent with those reported by a previous study, which was conducted using acoustic observation with an A-tag in the same area (Dede et al., 2013). In this study, I extracted the clicks only after visual confirmation. Most of the extracted clicks were at a closer range than the maximum detection range and each click train showed clear acoustic characteristics. The long-range propagation leads to the loss of the higher frequency component. The power spectrum of the delphinids would be shifted to the lower frequency side for the sound that travelled for several hundred meters. Distance dependence of the performance was not examined due to the limited visually confirmed data of families at a long distance in the present study. The proposed procedure of quantitative classification using visually confirmed ground truth data might be applied to target sounds of various biological sources.

Chapter 3

Density Estimation Using Acoustic Cues

3.1. Background

3.1.1. How to Estimate Population Density

Reliable estimation of population density is one of the most basic information to understand ecology and the status of conservation of wild animals. Today, there are several approaches to estimate animal density. The most commonly-used approach is based on mark-recapture or distance sampling. In mark-recapture approach, we find or attach unique ‘mark’ on an animal when it is captured. When we recaptured the animals from the same population, the proportion of firstly captured animals, which has marks to identify, is the key to estimate population density (Seber, 1982). This method is useful if animals can be easily captured or have natural marks for identification; however, either of catching or finding unique marks is difficult in some species including small cetaceans. The distance sampling is effective method for such elusive animals because it does not require either catching or finding a mark. In this method, the distance from the survey line or point to objects of interest, usually it is an individual or a cluster of animals, is used as key information for density estimation (reviewed by Buckland et al., 2001).

When a certain number of animals n was observed within randomly selected survey area a from the area A, and assuming all animals within area a was counted, the simplest formula to estimate animal density $\widehat{D}$ is written as Eq. (3.1). The density $\widehat{D}$ can

be converted the absolute abundance N in an area A as Eq. (3.1).

$$\widehat{D} = \frac{n}{a} \tag{3.1}$$

$$N = \widehat{D}A \tag{3.2}$$

Hereafter, all notation of variable are based on Buckland et al., (2001) as a principal citation.

3.1.2. Line Transect Sampling

When the observation was designed as a line in a survey area, the line transect distance sampling can be adopted. In line transect sampling, observers count the number of objects along survey line with the distance from the line to objects (Fig. 3.1). This method can cover large spatial area that is important to avoid sampling bias caused by the selection of survey area. The idea of the distance sampling is that the detection proportion of animals near the survey line is almost perfect, but it becomes lower depending on the distance from observer to objects. Therefore, in this method, we provide the detection proportion $\hat{P}_a$ within observation width w (or estimated Effective Stripe Width (ESW) $\hat{\mu}$. details are explained below) to estimate density $\widehat{D}$ as follows:

$$\widehat{D} = \frac{n}{2wL\hat{P}_a} = \frac{n}{2\hat{\mu}L} \tag{3.3}$$

Here the detection proportion is $\hat{P}_a$, the detected number of objects is n, the length of survey line is L, the one side survey width from the line is w and the ESW is $\hat{\mu}$.

$\hat{P}_a$ is estimated based on the detection function $\hat{g}(x)$ (Fig. 3.2). The detection function $\hat{g}(x)$ is the probability of detecting an object, given that it is at distance x from the random line (or point as mentioned below Point Transect Sampling section) (Buckland et al., 2001). Half-normal, hazard-rate and negative exponential distribution model are commonly used to fit the distance data to estimate $\hat{g}(x)$. After $\hat{g}(x)$ was estimated, $\hat{P}_a$ can be written by using $\hat{g}(x)$ and one side survey width w as Eq. (3.4). $g(0)$ is the detection probability at distance 0. Traditionally, it is assumed $g(0) = 1$ as the objects on line are perfectly detected. μ is the Effective Stripe Width (ESW) distance at which as many objects detected beyond $\hat{\mu}$ is as the missed objects within $\hat{\mu}$ (Eq. (3.4), Fig. 3.1).

$$\hat{P}_a = \frac{\int_0^w \hat{g}(x)\,dx}{wg(0)} = \frac{\hat{\mu}}{w} \tag{3.4}$$

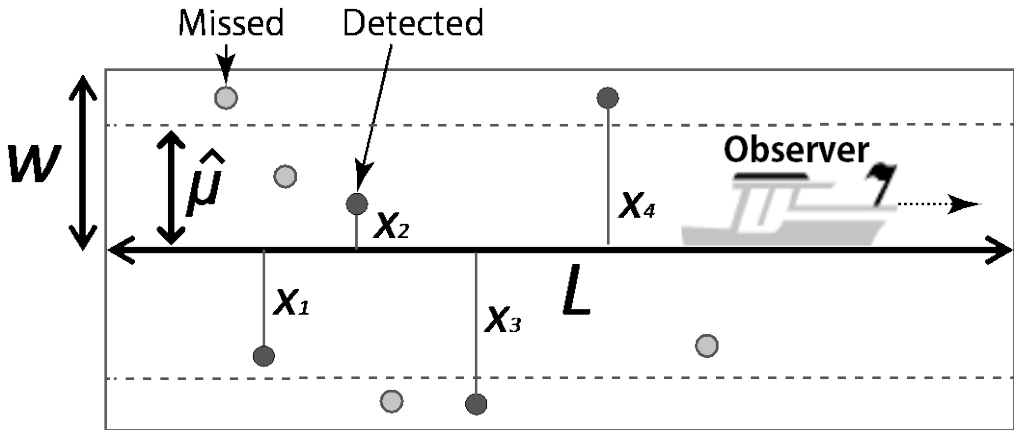

Fig. 3.1 The framework of the boat based line transect sampling. The observation was conducted on the survey line L with survey width w. Objects were detected at distance x_i. The Effective Stripe Width (ESW) $\hat{\mu}$ is the distance at which as many objects detected beyond $\hat{\mu}$ is as missed objects within $\hat{\mu}$.

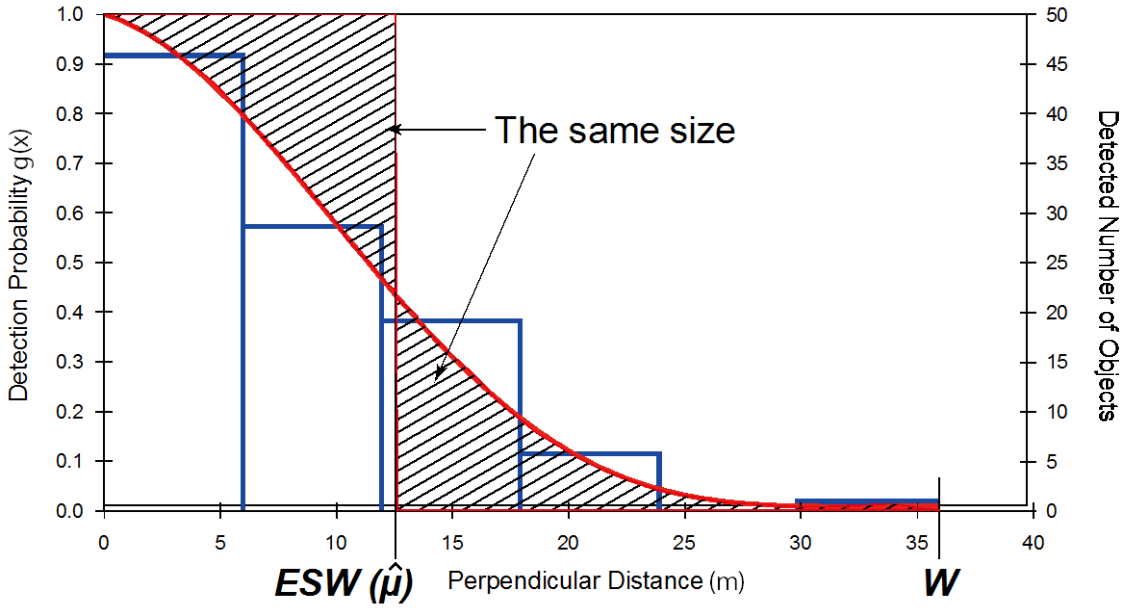

Fig. 3.2 The diagram of detection probability $\hat{g}(x)$ and Effective Stripe Width (ESW) $\hat{\mu}$. It was adapted from the sample data set provided with the software DISTANCE.

Recent rapid development of PAM for marine mammals provided the idea of using animal's sounds to density estimation. Both visual observation and trapping are difficult in the case of marine mammals; however, they frequently produce sounds underwater as mentioned in Chapter 1. In PAM, the recorder equipped on survey boat detects the unique sounds produced by small cetaceans. One consequent track of sounds is counted as the individual animal, and the distance from the survey line to the track is measured. This method is applied to density estimation of the animals which produce distinctive click sounds in relatively high signal-to-noise level, such as sperm whale *Physeter macrocephalus* or vaquita *Phocoena sinus* (Gerrodette et al., 2011; Hastie et al., 2003; Lewis et al., 2007). In these studies, the object of interests is individual animal therefore, the density estimation formula was identical with Eq. (3.3).

3.1.3. Point Transect Sampling

When the observation was designed as a point in the survey area, the point transect distance sampling can be adopted. The observation points are randomly selected in the survey area, and obtain the distance from the point to object (Fig. 3.3). This method is effective particularly when setting the transect line is difficult for some geographical reasons or for the habitat distributing as patch. The major advantage of this method comparing with the line transect sampling are the simplicity of collecting distance data. For line transect, we need to calculate the distance between object to line, but simply the distance between observer to object is required in the point transect sampling. However, this method may cause double count problem for mobile animals, such as small cetaceans, if the survey duration is not instantaneous. The idea of using distance to estimate the detection proportion $\hat{P}_a$ is the same as line transect. The density $\hat{D}$ is estimated as follows:

$$\hat{D} = \frac{n}{k\pi w^2 \hat{P}_a} = \frac{n}{k\pi \hat{\rho}^2} \tag{3.5}$$

$\hat{P}_a$ is estimated based on the detection function from the recorded distance the same as line transect Eq. (3.3); however, the survey area increases linearly with distance from observer unlike line transect sampling, namely, the number of objects increases at a distant than in close. Therefore, $\hat{P}_a$ for point transect sampling is estimated as Ep. (3.6). Instead of the survey area $2wL$ in the Eq. (3.4), survey area was calculated

as $k\pi w^2$ or $k\pi\hat{\rho}^2$. k is a number of observation points (if point transect sampling was conducted at one point, $k = 1$), and w is survey radius, and $\hat{\rho}$ is the Effective Detection Radius (EDR) which is the same sense with ESW $\hat{\mu}$.

$$\hat{P}_a = \frac{2\pi \int_0^w x\hat{g}(x)\,dx}{\pi w^2 g(0)} = \frac{\hat{\rho}^2}{w^2} \tag{3.6}$$

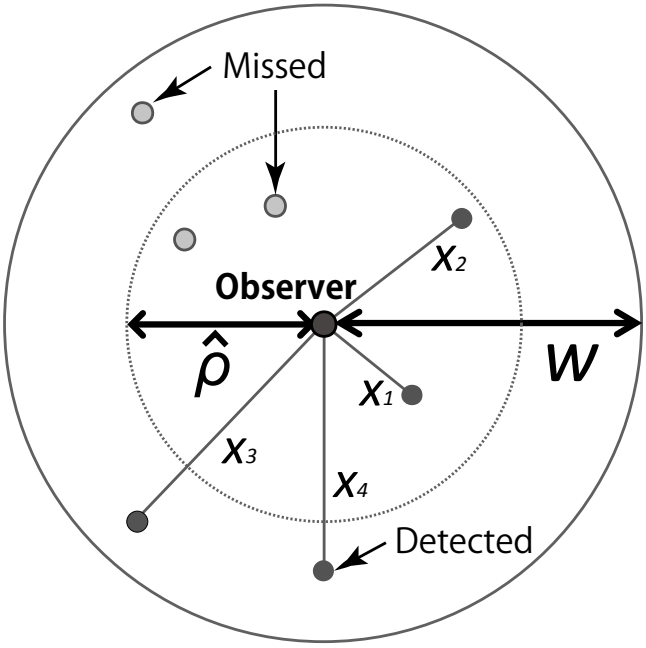

Fig. 3.3 The framework of the point transect sampling. The observation was conducted on the point with survey width *w*. Objects were detected at distance x_i. The Effective Detection Radius (EDR) $\hat{\rho}$ is the distance at which as many number of objects detected beyond $\hat{\rho}$ is as that of missed within $\hat{\rho}$.

If we can count the number of sounds but not the number of animals in PAM, we count the individual (or cluster of) sounds as the object of interests, then, need to convert the animal density by dividing by the vocalization rate of that individual/cluster of sounds during survey period. In case we detect the sounds instead of individual animals, false positive detections of noises cannot be avoided. Although there are false positive and false negative detections caused by direct visual observation, many kinds of biological or anthropogenic noise, and recently, the sounds from other non-target small cetacean species raise the proportion of false positive detections and false

negative detections in PAM. Therefore, the false positive detection should be taken into account to use acoustic cues for object of interests. The point transect density estimation formula by using acoustic cues can be written as Eq. (3.7) it was proposed by Marques et al., (2009), and modified for click trains of small cetaceans by Kimura et al., (2010).

$$\widehat{D} = \frac{n(1-\hat{f})\,\alpha}{\pi w^2 \hat{P}_a T \hat{r} \hat{c}} = \frac{n(1-\hat{f})\,\alpha}{\pi \hat{\rho}^2 T \hat{r} \hat{c}} \tag{3.7}$$

The parameters of the false positive proportion (i.e. false alarm rate) $\hat{f}$, true positive proportion (i.e. true positive rate) $\hat{c}$, coefficient of group size and the number of click trains produced in a unit time α which is 1 if the number of objective sounds linearly increase with the group size, survey period *T*, and the vocalization rate per unit time $\hat{r}$, were added in Eq.(3.5) to use acoustic cues obtained by PAM as the object of interests.

The vocalization rate $\hat{r}$ can be estimated by one of three approaches; conduct acoustic bio-logging, refer the published study, or track the animal by acoustic arrays (Marques et al., 2009). In bio-logging experiment, acoustic tag is attached an individual animal so that tag can record all the sounds during it is attached. Ideally, the vocalization rate would be obtained from the representative target animal of density estimation. Moreover, it has the same parameter, such as season, time of day, sex and the same extent of sexual maturation with target animal is ideal (Marques et al., 2009) if it potentially influence the vocalization rate. In acoustic tracking, the vocalization rate should be obtained when the animals are very close to a recorder that cannot miss any sounds from animal.

Marques et al., (2009) provided Eq. (3.5) with showing an example of Blainville's beaked whale *Mesoplodon densirostris* density. Beaked whale is one of the species which make long deep dives, and considered it is relatively difficult to observe visually. However, it is known that this species produce distinctive high frequency clicks during foraging dives (Johnson et al., 2004; Madsen et al., 2013). This distinctive click was used as the acoustic cues. After this estimation formula was proposed, it has been applied to other species such as minke whale *Balaenoptera acutorostrata* (Martin et al., 2013), Yangtze finless porpoise (Kimura et al., 2010) and harbor porpoise (Kyhn et al., 2012).

3.2. Cue Counting Line Transect with False Discrimination Rate

As mentioned in Chapter 2, species identification becomes strongly anticipated technique in PAM. However, quantitative classification of species ratio has not been feasible so far. Recent increasing number of species identification attempts (e.g. Gillespie et al., 2013; Oswald et al., 2007; Roch et al., 2007) raise the issue of false discrimination in small cetacean species. Some cetacean species have very specific call, such as boing sounds from minke whales (Rankin and Barlow, 2005) or star-wars sounds from dwarf minke whales (Gedamke et al., 2001), which can be recognized easily and be used as explicit acoustic cue for density estimation (Martin et al., 2013). However, it is not the case for many small cetacean species. The major reason is that the ground truth of the species besides acoustic monitoring is not available in many cases. Classified acoustic feature should be associated with independently confirmed species identification. Since it is impossible to develop the species classification method with 100% accuracy, we need to consider the effect of false discrimination that must influence the density estimation.

The effect of uncertainity on species classification to the estimatied density has been augued in some studies. For example, how the false discrimination affects the variance of estimated density for marine mammals in PAM (Caillat et al., 2013), or in aerial line transect survey (Conn et al., 2013) based on simulations. Conn et al., (2014) and Johnston et al., (2015) estimated the population density with considering the false discrimination error for ice-associated seals and sea birds, respectively. However, until very recent as Johnston et al., (2015) stated as well, there are not much described studies which account for species uncertainty in estimates of abundance. Moreover, there is no explicit methodology to consider the false discrimination using PAM. As described in Chapter 2, the quantitative descrimination of species has been partially solved in our study and this method will be appried for following chapters as well.

Besides the acoustic line and point transect methods have been developed as described previously, density estimation using acoustic means is not always possible due to practical issue raised in the field observation. Line transect is able to provide

abundance of target species in wide area (Gerrodette et al., 2011; Hastie et al., 2003; Lewis et al., 2007). It is essential to count the number of an individual or cluster of animals to apply this methodology, therefore, these studies target at the species which tend to have small group size or have loud distinctive acoustic cues. However, individual discrimination is not always possible using acoustic means even on a moving platform especially when animals make groups or the signal-to-noise ratio is low as in the case of many biological or anthropogenic noises contaminated in PAM.

On the other hand, cue based method to estimate density of target animals is employed within an observable area. However, the coverage area by single acoustic detector is quite limited. For reliable estimation in broad survey area, we need several detectors. It is costly especially in click-based density estimation for small cetaceans, because high frequency sounds, such as clicks, attenuate rapidly.

The idea I propose here is to merge two methods and add parameter of false discrimination of species to estimate density of certain small cetacean species. Using a moving platform similar to the line transect method, but estimating density by acoustic cue counting, which was used in point transect method.

I propose the new survey design and density estimator for PAM. To cover the large spatial area with low cost, the cue counting line transect density estimation is appropriate. The basic cue counting density estimation method was developed to estimate population density of whales using blow (Hiby and Ward, 1986). The idea of blow based cue counting is the same as the density estimation in the point transect sampling using acoustic cues. In the case of PAM, we need vocalization rate to convert the detected number of acoustic cues to density, similarly, we need blow rate to convert the detected number of blows to density in blow based cue counting. The difference between them is the type of survey. In blow based cue counting, the survey was designed similar to line transect sampling yet theoretically it is close to point transect. The observer scans a sector of angle φ ahead of the boat. Distances are estimated to detected cues within the sector (Buckland et al., 2001; Fig. 3.4).

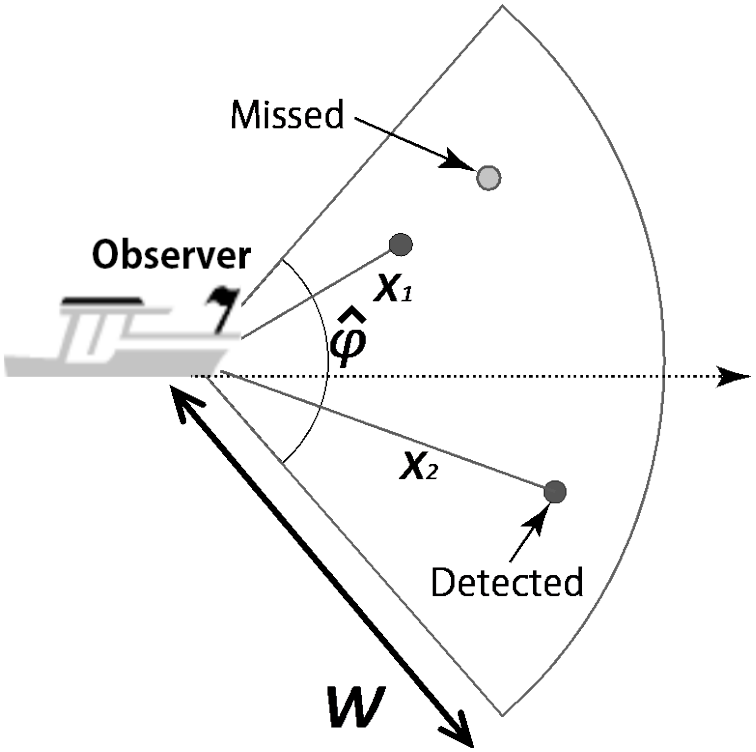

Fig. 3.4 The framework of cue counting. The observation was conducted sector of angle φ with survey width *w*. Objects were detected at distance x_i.

In the case of PAM, the story is more simple since the searched radius φ is 2π. Therefore, the same formula with Eq. (3.7) can be used also for cue counting line transect. By using this survey design for acoustics, I can solve the inconvenience of counting an individual/cluster of animals in line transect sampling, and of the limitation of special coverage in point transect sampling (Fig. 3.5). The problem of false discrimination in species is taken into account together by adding the parameter of false positive proportion (false alarm rate) and false negative proportion. The formula is proposed as follows:

$$\widehat{D} = \frac{n\left(1-\hat{f}_1\right)\left(1-\hat{f}_2\right)\alpha}{\pi\hat{\rho}^2 T\hat{r}\hat{c}_1\hat{c}_2} \tag{3.8}$$

Here, the detected number of sounds *n*, proportion of false positive detection and discrimination $\hat{f}_1$ and $\hat{f}_2$, respectively, group effect α, survey area πw^2, effective detection radius $\hat{\rho}$, survey period *T*, the vocalization rate per unit time $\hat{r}$ and proportion of true positive detection and discrimination $\hat{c}_1$ and $\hat{c}_2$, respectively.

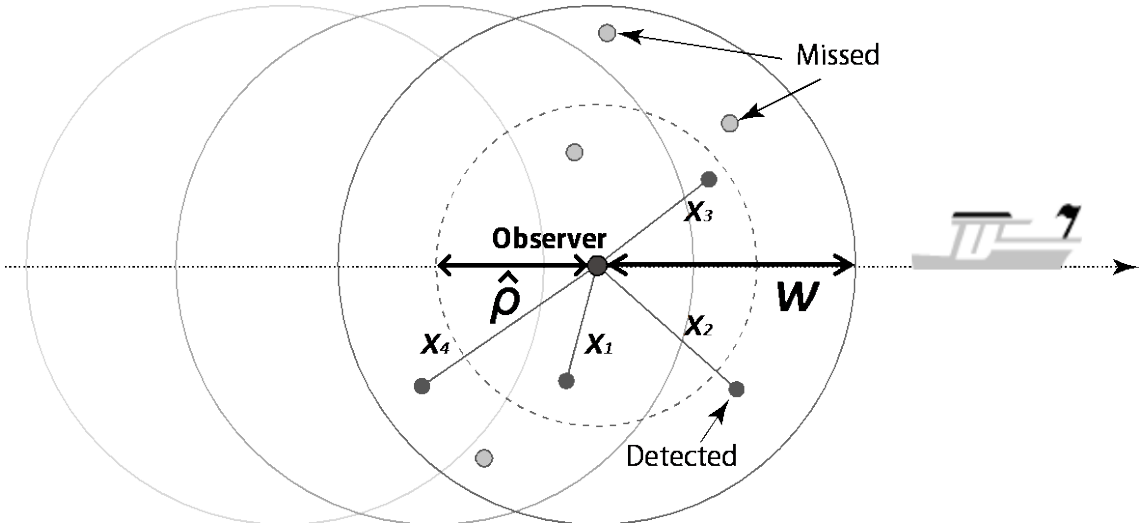

Fig. 3.5 The framework of cue counting line transect. Objects were detected at distance x_i. The Effective Detection Radius (EDR) ρ is calculated from detection probability as well as point transect.

Chapter 4

Seasonal Change in Presence and Acoustic Behavior

4.1. Introduction

The Istanbul Strait (Bosphorus), in Turkey, is an important corridor between the Black Sea and Marmara Sea for many marine organisms (Fig. 1.3) (Öztürk and Öztürk, 1996). It is approximately 31 km long, 0.7–3.5 km wide, and 35–110 m deep (Yüce, 1996). It is well known that three small cetacean species are observed in this strait, the harbor porpoise *Phocoena phocoena*, bottlenose dolphin *Tursiops truncatus*, and short-beaked common dolphin *Delphinus delphis* as detailed described in Chapter 1.

When more than two small cetacean species occupy a similar habitat, many types of interspecies relationships, such as aggressive behavior, the constitution of mixed species groups, and ecological separations in habitat usage, have been observed (reviewed by Bearzi 2005). Several types of ecological separation within sympatric small cetacean species have been reported, mainly based on dietary or geographical features. Dietary separation includes not only a difference in prey species, but also a difference in the time of day or season for foraging. Since small small cetaceans are opportunistic feeders that prey on abundant species, they change feeding strategies to adjust the trade-off between energy intake and consumption required for prey capture. Resource competition between sympatric small cetaceans might be one of the important reasons for habitat separation.

When the habitat separation does not seem to be successful, direct conflict and aggressive behavior between sympatric species has been reported. The aggressive behavior of bottlenose dolphins toward harbor porpoises in the Moray Firth, Scotland, is one of the most well-known examples of sympatric species conflict (Ross and Wilson, 1996). Attack by bottlenose dolphins has been proved to cause the death of stranded harbor porpoises in some cases (Jepson and Baker, 1998; Ross and Wilson, 1996). Many previous studies investigated the reason for such aggressive behavior, suggesting an overlap in prey (Spitz et al., 2006) or a connection with infanticide (Patterson et al., 1998); however, the explanation remains unknown.

These studies of interspecies relationships were mostly based on visual observations of surface behavior, which can only be a snapshot of actual habitat use. Some recent studies have focused on habitat partitioning between sympatric harbor porpoises and bottlenose dolphins based on acoustic observations. These studies indicated that it is rare for both species to appear simultaneously (Simon et al., 2010) and that harbor porpoises became acoustically inactive or left the monitoring area when bottlenose dolphins were detected (Jacobson et al., 2015). However, habitat sharing among multiple species of small cetaceans based on continuous, long-term observation remains largely unknown. In addition, it might be quite variable from place to place, since prey species or basic ecology differs even within the same small cetacean species. An understanding of local sympatric ecology is an important source of clues about possible reasons for the aggressive behavior, as well as for basic information on niche partitioning.

As already mentioned, aggressive behavior from bottlenose dolphins to other small cetaceans has been reported in several regions. Although it has been visually confirmed that both harbor porpoises and delphinids appear in the same area simultaneously, such aggressive behavior has never been reported in this strait. This may indicate that there are some mechanisms by which they avoid direct conflict. In this study, fixed passive acoustic monitoring was employed to observe the presence, location, and echolocation behavior, represented as inter-click intervals (ICIs), between harbor porpoises and the other two delphinids in a focal area. The ICI, the duration between clicks in a click train, varies with the distance from an animal to a target. It is relatively

stable in harbor porpoises, at around 60 ms, compared with bottlenose dolphins, but both species can adjust it based on the target range (Au, 1993; Villadsgaard et al., 2007; Wisniewska et al., 2012). Dede et al., (2013) conducted a continuous passive acoustic observation in the strait without identifying the species, and detected large numbers of click trains in the middle of the strait during the nighttime in March and April. This study suggested seasonal and diurnal changes in the occurrence of small cetaceans; however, the contribution of each species remains unknown. Observation of each species separately in the strait is needed to understand the difference in habitat use among species.

Recent findings and advances in technology enable us to distinguish between the harbor porpoise and delphinids based on echolocation sounds. Harbor porpoises produce narrow-band high-frequency (NBHF) clicks that have a peak frequency of around 130 kHz (Villadsgaard et al., 2007). On the other hand, many delphinids species, including bottlenose dolphins and short-beaked common dolphins, produce broadband clicks that have similar intensities from low to high frequency (*e.g.*, 23 kHz to 134 kHz for bottlenose dolphins) (Au, 1993). The intensity ratio of two frequency band components at 130 kHz and 70 kHz is used to identify these two families of small cetaceans quantitatively. Using the long-term acoustic dataset obtained from the focal area in the Istanbul Strait, I aimed to reveal how these species share the same area simultaneously. In this study, I focused on elucidating (1) the differences in the occurrence of harbor porpoises and delphinids throughout the year; (2) the differences in detected location and diel preference; and (3) the differences in echolocation behavior represented by inter-click intervals (ICIs). These data can be used as an index of underwater behavior. Based on the results, I discuss several factors causing differences in habitat use by multiple species appearing in the same location.

4.2. Materials and Methods

4.2.1. Acoustic Event Recorder with Species Classification

I deployed a stereo passive acoustic monitoring device, A-tag (ML200-ASII; Marine Micro Technology, Inc., Saitama, Japan), near the narrowest point of the

Istanbul Strait (Fig. 1.3 and 2.1). A-tag is an event data-logger that records sound pressure and the time difference in sound arrival (TDOA) between two hydrophones when the sound exceeds a preset threshold (138 dB peak-to-peak re 1 µPa). It does not record the waveform itself. Each hydrophone was sensitive at a different frequency; the first was most sensitive at 130 kHz and the second at 70 kHz (Fig. 2.1). A passive two-pole band pass filter of 55–235 kHz, which includes the peak frequency of harbor porpoises and delphinids echolocation signals, was used (Au, 1993; Villadsgaard et al., 2007). Differences in the maximum sensitivity of each hydrophone enable us to distinguish harbor porpoises and delphinids. The sound pressure ratio of the two frequencies (130 and 70 kHz, referred to as the two-band ratio hereafter) shows different distributions for harbor porpoises and delphinids. For family discrimination, I first obtained echolocation clicks with visual species confirmation to calculate the two-band ratio. Second, I calculated matched and unmatched species identification events by acoustical means by comparing with visual observation, which provided correct detection and false alarm rates. For detailed methodology of species discrimination, please refer to Chapter2 and (Kameyama et al., 2014).

The first and second hydrophone of the A-tag are set 600 mm apart. The first hydrophone is the trigger to record TDOA with a resolution of 1084 ns. The mechanical dynamic range is ±511 counts (10 bits) and the actual maximum and minimum counts are approximately ±403 counts, which correspond to ±90°, based on the approximate sound speed and the baseline length of the stereo hydrophones. The sound speed in the upper layer in the strait is estimated as 1486 m s^{-1} by the UNESCO algorithm, assuming the average salinity is 17.5 and temperature is 15 °C (Chen and Millero, 1977). The counts were converted to degrees. The A-tag was deployed in order to maintain the baseline parallel to the shoreline; therefore, negative degrees indicate that the sound source is in a southern direction from the device (the Marmara Sea side), and positive degrees indicate it is in a northern direction (from the direction of the Black Sea). The received sound pressure and TDOA were stored in the flash memory of the A-tag every 2 ms. For more details about A-tag, please refer Chapter 1.

4.2.2. Data Collection

The A-tag was deployed on the European side of the middle of the Istanbul Strait (41°05′835″N, 29°03′264″E; Fig. 1.3) continuously from January 2010 to December 2011, except for October 2011 for machine maintenance. The distance from the A-tag to the opposite coast was approximately 840 m. An iron pipe and a metal basket to protect the body of the A-tag were fixed beside a pier located on the bank of the strait. The A-tag was positioned at a depth of approximately 90 cm (35 cm above the bottom) (Fig. 2.1). The change in tide level was under 34 cm in this area (Alpar and Yüce 1998). This was above the depth of the A-tag, which was not exposed to air at any time. The depth at the deployed point was 125 ± 34 cm, depending on the tide level.

I assumed simplest spherical spreading to estimate the maximum detection range of clicks because complex bathymetry and oceanography prevents to know reliable parameters for shallow water propagation modeling in this region. In addition, spherical spreading model is robust and reasonable if I only need the rough approximation of propagation loss (Urick 1996). The Istanbul Strait consists of two layers of water flow, an upper layer coming from the Black Sea with low salinity and a lower layer coming from the Marmara Sea with high salinity. There is a sharp intermediate transition layer at around 35 m near the monitoring area (Tarkan et al., 2005). Since animals mostly stay shallower than 35 m even though they are capable of diving deeper (Otani et al., 1998), I used the average salinity and temperature of the upper layer for estimation. Each parameter was calculated using the following parameters: maximum source sound pressure level (Source SPL), which was estimated at 227 dB peak-to-peak re 1 µPa for bottlenose dolphins (Simard et al., 2010) and 205 dB peak-to-peak re 1 µPa for harbor porpoises (Villadsgaard et al., 2007); the minimum received sound pressure level of the present A-tag (Received SPL: 138 dB peak-to-peak re 1 µPa); and the absorption coefficient of the Istanbul Strait (α). Assuming the average salinity of the upper layer was 17.5 and the temperature was 15 °C, α was estimated at 0.026 dB m^{-1} for 130 kHz and 0.013 dB m^{-1} for 70 kHz using the Francois and Garrison model (Francois and Garrison, 1982a, 1982b). The maximum detection range d was estimated using Eq. (2.1).

The maximum detection range for harbor porpoises was calculated at 630 m,

while that of bottlenose dolphins was more than 1,000 m, which exceeded the width of the strait at the observation point. It is important to note that the maximum detection distance is for the loudest clicks of an on-axis beam. The detection distance of off-axis beams or low source level sounds could be shorter than these estimated values.

4.2.3. Off-Line Data Analysis

I applied an off-line filter using a custom-made script designed for the Igor Pro 6.2.2 platform (WaveMetrics, Inc., Oregon, USA) to exclude false detections that may be caused by contamination from background noise. The function of the filter is to eliminate surface or bottom reflections, ship noise, or overlapped sonar signals of two or more animals produced simultaneously. This filter has three steps. In the first step, I eliminated the sounds recorded within 2.5 ms, which were likely reflected sounds from the surface or the bottom instead of direct path sounds. In the second step, I selected for pulse trains with small variation in the ICIs. Porpoises and delphinids produce clicks as a sequence of pulsed sounds as trains with relatively regular ICIs, unlike background pulsed noise (Au, 1993). A separation between click trains was defined as 200 ms from the previous click (Akamatsu et al., 2007). The first selection criterion for click trains is that there should be a minimum number of pulses in a click train—here designated as six or more (Akamatsu et al., 2010b; Kimura et al., 2010). Pulse sequences containing a small number of clicks were not considered, as it is hard to distinguish these from occasional mechanical noise. The second criterion is that the coefficient of variation of the ICIs in a click train should be smaller than 0.3, which ensures the regular change of ICIs in a train (Kimura et al., 2010). Finally, all data were checked manually to exclude heavy ship noise, which was not excluded by auto-filtering.

To distinguish each family, I used a two-band ratio of 130 kHz and 70 kHz. When the clicks of harbor porpoises and delphinids are mixed in the same period, the two-band ratio shows a bimodal distribution in which each peak corresponds to a species. The appropriate threshold to identify each family was estimated based on the comparisons with ground truth visual observations with the two-band ratio (Kameyama et al., 2014). The threshold was estimated on a monthly basis. When almost all detections were from either porpoises or delphinids, the distribution did not show a bimodal shape. Therefore, it was difficult to distinguish the minority family in such

cases. In particular, when more than 82% or less than 26% of detections were estimated to be from one family, the classification contained a more than 20% false positive error (Kameyama et al., 2014). Thus, to better focus on the aim of this study, which was to examine the habitat sharing by multiple species, I excluded the month with a more than 20% false positive error from the analysis.

I calculated detection positive minutes (DPMs) to analyze seasonal and diel changes in detections for both harbor porpoises and delphinids. A DPM is a minute in which at least one click train was detected. The detected number of DPMs per day or DPMs per hour, the sound source direction, and the average ICIs were calculated for each detected click train. These values were compared between months and between families. Seasons were classified as spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February). To determine the effect of diel variation on the number of DPMs, I defined the four diel phases in a day as follows: morning (0400–0800 h), daytime (0800–1700 h), evening (1700–2100 h), and night (2100–0400 h). The morning covered dawn and sunrise, evening covered dusk and sunset, respectively. The times of sunrise and sunset were obtained from the Astronomy Laboratory, Boğaziçi University, Istanbul, Turkey (http://www.koeri.boun.edu.tr/astronomy/dogus-batis/Istanbul.htm).

All analyses were performed using R 3.0.2 (R Development Core Team 2014) and Igor Pro 6.2.2 (WaveMetrics, Inc., Oregon, USA).

4.3. Results

4.3.1. Seasonal Trend of Acoustic Encounters

Acoustic monitoring was conducted over a 24-month period from January 1, 2010 to December 31, 2011, except during October 2011 (Fig. 4.1). Both harbor porpoises and delphinids were present in the focal area simultaneously, as shown in Fig. 4.1, although seasonal variations in the number of acoustic detections were observed.

The number of DPMs per day varied significantly among months for both harbor porpoises and delphinids (Kruskal–Wallis test, porpoise: $H = 441.47$, $P < 0.0001$; delphinids: $H = 321.1$, $P < 0.0001$). The largest number of click trains detected per day

was 84.06 ± 98.89 (mean ± SD) in December 2011 for delphinids and 86.03 ± 144.36 in May 2011 for harbor porpoises.

As mentioned in the Materials and Method, I excluded the months with a more than 20% false positive rate. In this study, I excluded the porpoise-classified data from January, February, November, and December in 2010, and January, February, August, September, October, and December in 2011 (shaded area in Fig. 4.1). Therefore, there are no winter data for harbor porpoises in the results.

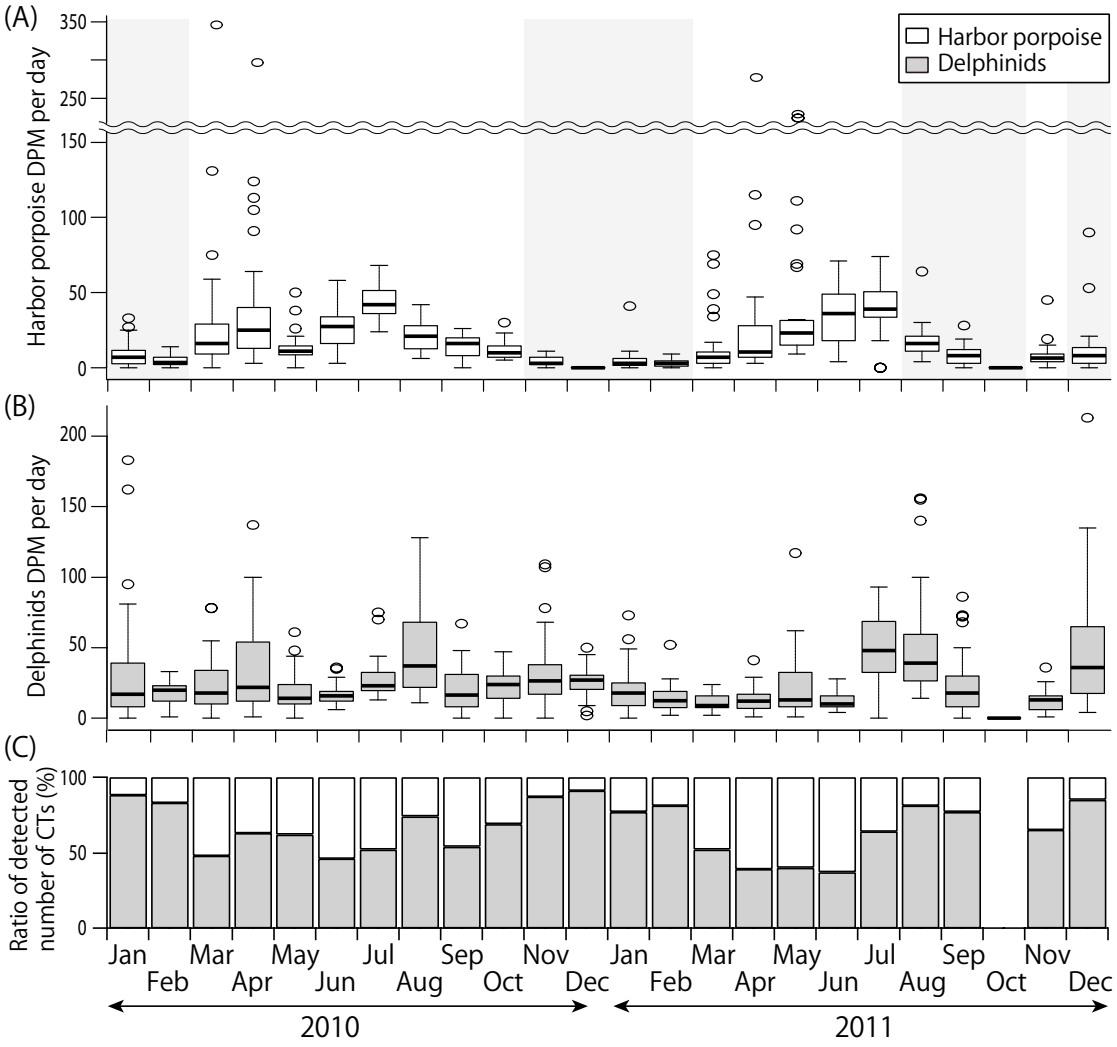

Fig. 4.1 The detected number of DPM per day (mean ± SD; (A) and (B)) for harbor porpoise (A) and delphinids (B). (C) is the ratio of detected number of click trains (CTs) in each month. Collection of data was continuous except for October 2011.

4.3.2. Localization

The sound source direction showed a southward localization from the monitoring station only for harbor porpoises in spring (Fig. 4.2). In total, 78.9% of the detected click trains of harbor porpoises came from the south in spring. This location-biased distribution was not clear in summer and autumn; 33.4% of the detected click trains of harbor porpoises came from the south in summer and 45.7% in autumn.

Delphinids did not show a specific localization, regardless of the season (Fig. 4.2); 59.0%, 53.7%, 58.7%, and 58.8% of click trains came from the south in spring, summer, autumn, and winter, respectively.

4.3.3. Diel Presence Pattern

Hourly variations in DPM are shown in Fig. 4.3. There were fewer DPMs for harbor porpoises during the morning than in other periods in both spring and summer (Kruskal–Wallis test with Dunn's test, $P < 0.01$) and fewer detections in the morning than in the daytime and evening in autumn (Kruskal–Wallis test with Dunn's test, $P < 0.01$). However, the diel change in DPMs was not clear, especially in summer and autumn (Fig. 4.3). On the other hand, delphinids showed a nocturnal presence pattern in all seasons (Fig. 4.3). The number of DPMs was significantly larger during the night than in the other periods in spring, summer and autumn (Kruskal–Wallis test with Dunn's test, $P < 0.01$).

4.3.4. Inter-Click Interval

Harbor porpoises used short ICIs with median values of 40.6 ms in spring (Fig. 4.4). However, the median extended to 96.8 ms and 109.5 ms in summer and autumn, respectively. Delphinids did not show such a drastic change between the seasons. The median ICI was 64.0 ms in spring and 68.3 ms in summer (Fig. 4.4). It increased in autumn and winter to 86.3 ms and 88.8 ms, respectively.

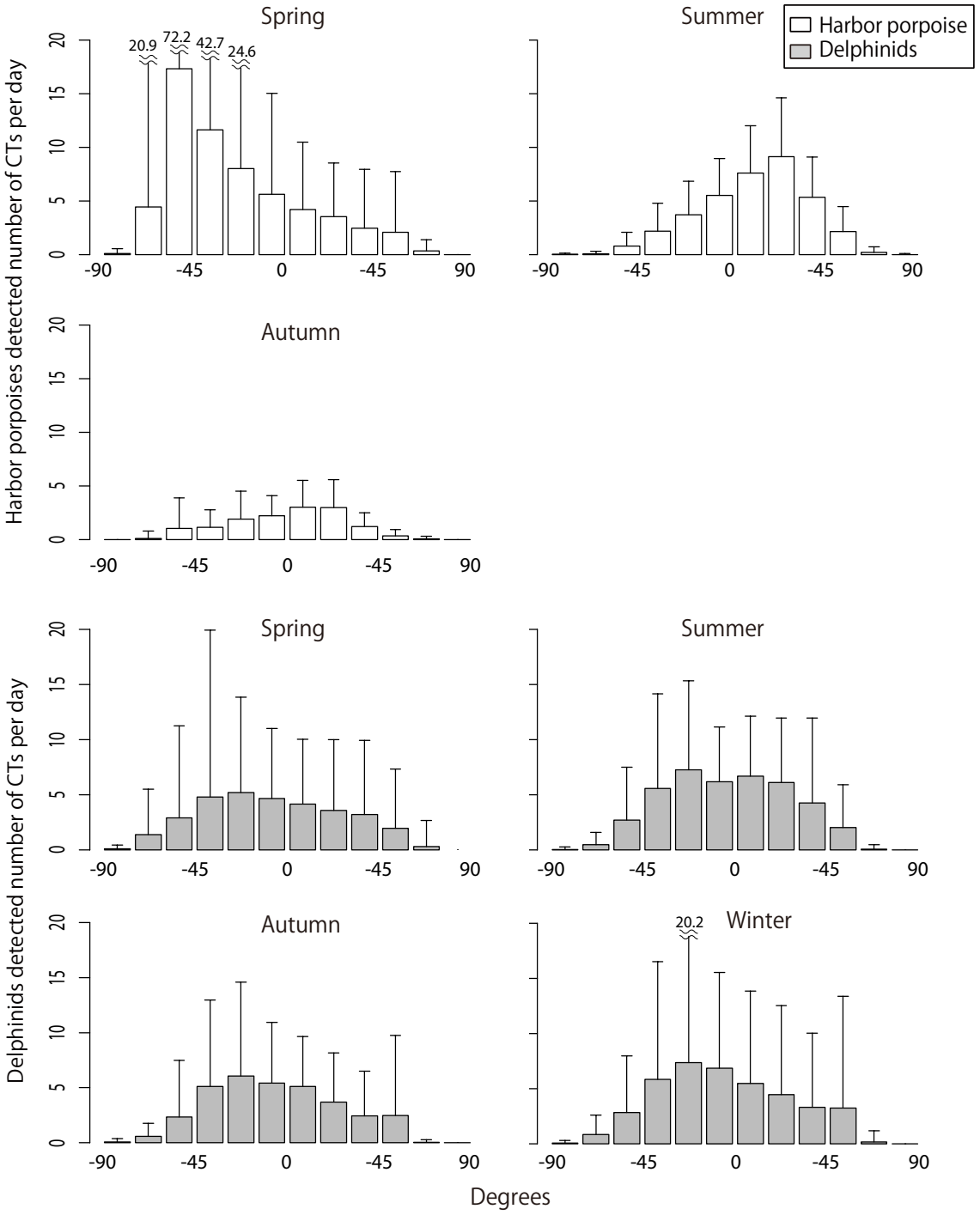

Fig. 4.2 The detected number of CTs per day in each 10 degree sector (mean ± SD). The minus degrees indicate the Marmara Sea side and the plus degrees indicate the Black Sea side. Exact ±90 degree indicates coastal line.

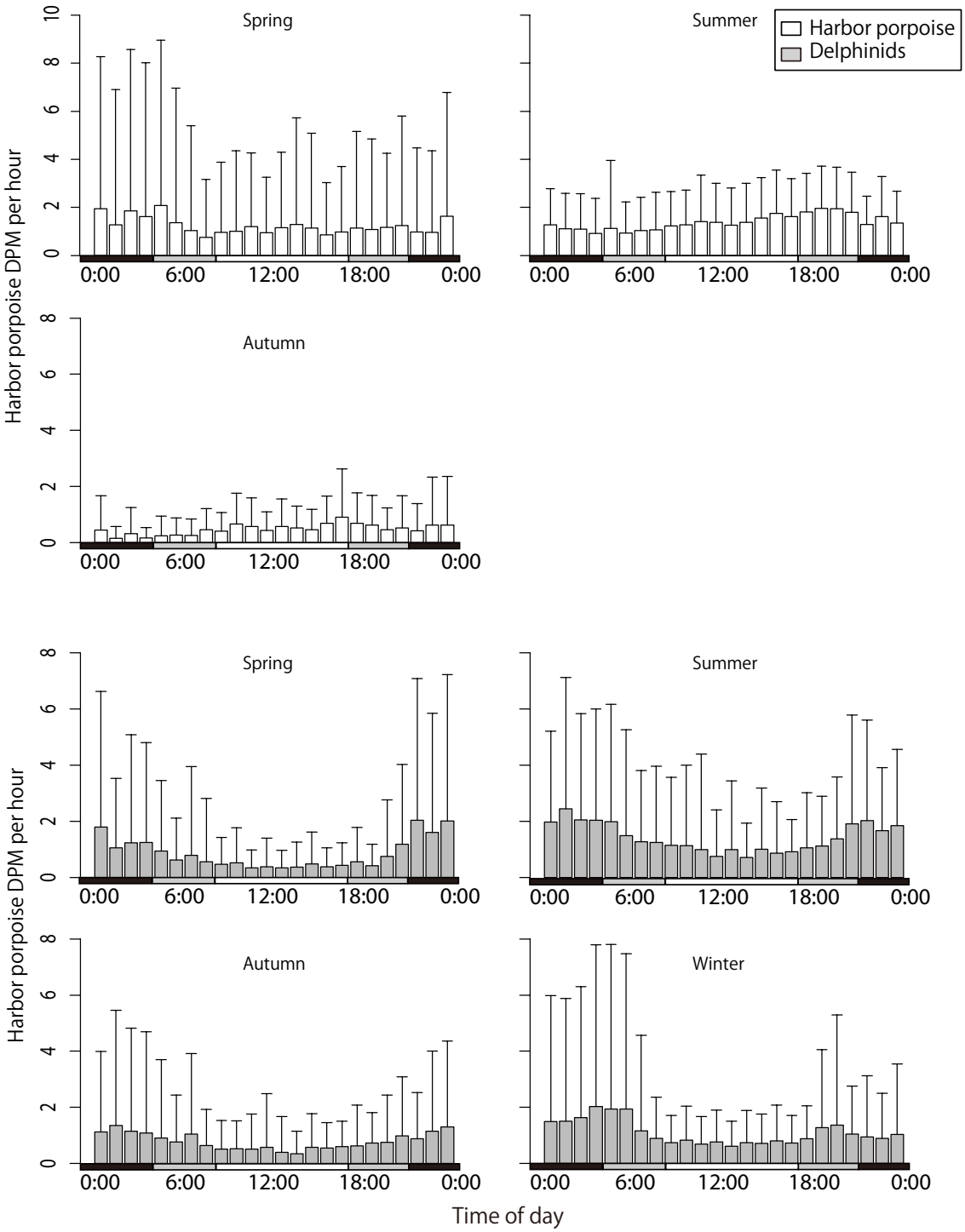

Fig. 4.3 The detected number of DPM per hour. Black, gray, and white bars on x-axis correspond to morning (0400–0800 h), daytime (0800–1700 h), evening (1700–2100 h) and night (2100–0400 h) respectively.

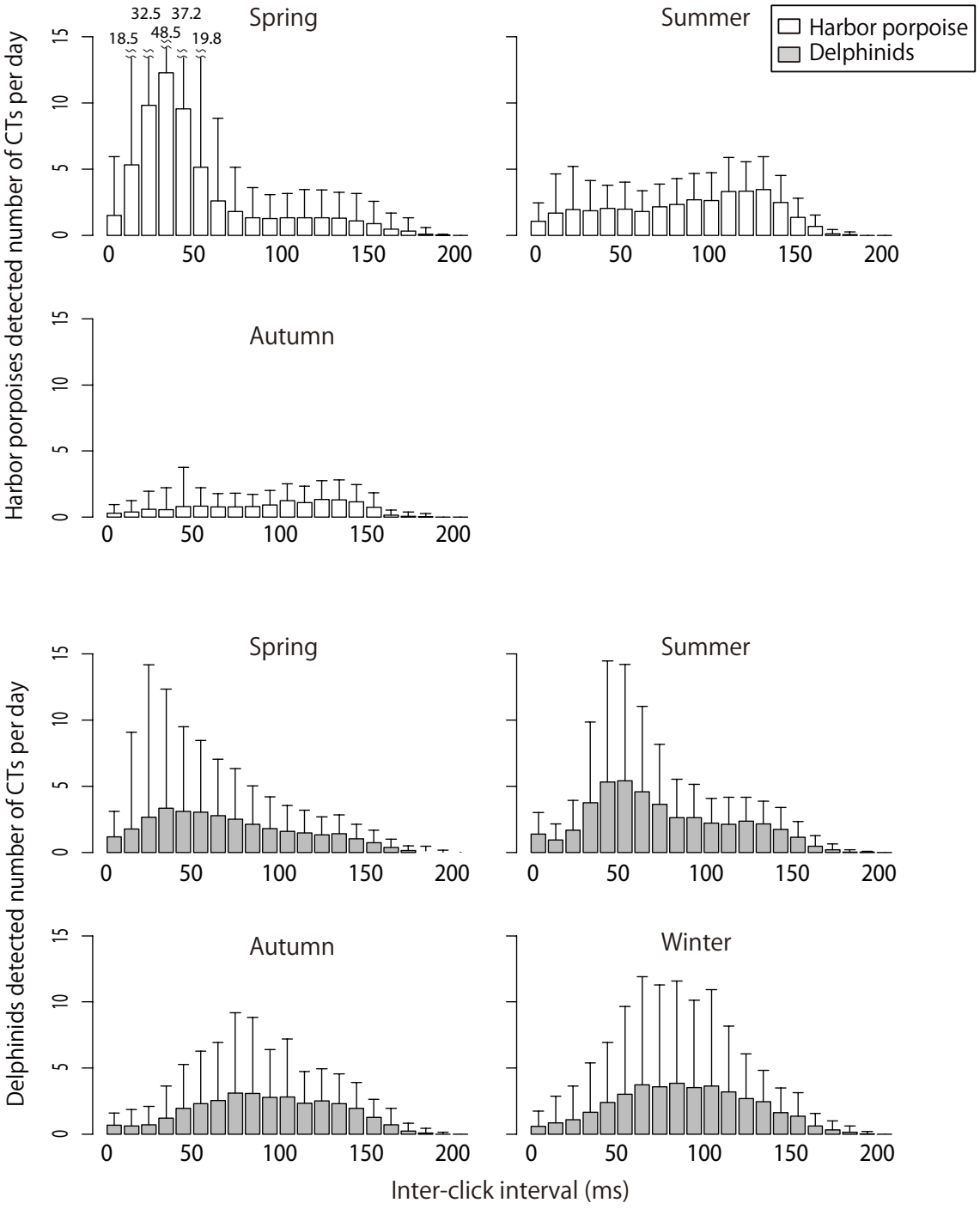

Fig. 4.4 The detected number of CTs per day with each ICIs (mean ± SD).

4.3.5. Summary of the Results

Two families of small small cetaceans, harbor porpoises and delphinids, were present at the same time during spring, summer, and autumn, especially, delphinids were detected every month during the 2-year monitoring period. In winter, delphinids were dominant and the detection of porpoises was very rare or almost none. Only in spring harbor porpoises localized south of the monitoring station and used short ICIs without showing a clear diel presence pattern. In summer and autumn, porpoises did not show clear localization and used longer ICIs without a clear diel presence pattern. Delphinids appeared in all directions and used relatively short ICIs with a clear nocturnal presence pattern in both spring and summer. The ICIs were relatively longer in autumn and winter, but localization and diel presence patterns were similar to those in spring and summer.

4.4. Discussion

I successfully conducted an almost continuous, 2-year-long, multi-species acoustic observation. my study revealed significant differences in the number of DPMs of both harbor porpoise and delphinids throughout the year (Fig. 4.2). The difference in DPMs might reflect the differences in the vocalization rate or abundance. Previous studies have suggested that the inter-individual variation in vocalization rate is larger than the diel variation (Kimura et al., 2013; Linnenschmidt et al., 2012). In addition, DPM reduced the individual bias in vocalization rates than the number of vocalization itself. Therefore, the difference in DPM probably reflects the difference in abundance of animals in the monitoring area. This difference of occurrence between families was not detected by Dede et al., (2013) because the authors did not focus on it, moreover, quantitative acoustic family discrimination was not possible at the time. My findings suggest that each species uses this focal area in different time and purpose, in spite of their sympatric existence.

Previous studies conducted in the Istanbul Strait by shipboard (Öztürk et al., 2009) or land-based theodolite (Baş et al., 2014) visual observations of the entire strait showed that harbor porpoises and bottlenose dolphins were sighted more frequently

from spring to early summer (April to May); that harbor porpoises are the dominant species observed in the middle of the strait and there are only few sightings of delphinids, especially near our monitoring area (Öztürk et al., 2009); and that a high density of bottlenose dolphins was observed within our monitoring area only in summer (Baş et al., 2014). The results of the present study are consistent with harbor porpoises being detected most frequently from spring to summer, and being the dominant species observed in the middle of the strait in spring to early summer. However, our data showed a large number of DPMs for delphinids in spring as well. A possible reason for this mismatch between the previously presented data and the data presented herein is the lack of information from the nighttime. The number of DPMs for delphinids was significantly higher in the nighttime than in other diel phases, On the other hand, harbor porpoises were detected regardless of the time of the day (Fig. 4.3). It is not possible to detect this difference in the diel presence pattern only by visual observations.

4.4.1. Habitat Use by the Small Cetaceans

The click trains detected from harbor porpoises were strongly localized in a southern direction from the monitoring station in spring, but the localization was not observed in delphinids (Fig. 4.2). These results suggest that the southern area is heavily used only by harbor porpoises and in spring. The previously reported median value of the ICIs of harbor porpoises was around 60 ms in both captive experiments and wild observations (Teilmann et al., 2002; Villadsgaard et al., 2007). In this study, the harbor porpoises used short ICIs, with a median of 40.6 ms, in spring, and longer ICIs in the other seasons (Fig. 4.4). On the other hand, delphinids used similar ICIs in spring and summer. It is known that the two-way sound travel distance within a click train relates to the maximum sensing distance by echolocation (Au, 1993). Particularly during the exhibition of feeding behaviors, the ICIs are reduced depending on the distance between animals and their target prey (Akamatsu et al., 2010b). Taking these factors into account, the results suggest that only the harbor porpoises were primarily feeding in the sector south of the monitoring station, regardless of time of day, in spring.

The delphinids did not localize in any direction and appeared during the evening or nighttime. In addition, they used longer ranges of sonar than the harbor porpoises in all seasons. Baş et al., (2014) indicated that the largest number of sightings of bottlenose

dolphin occurred during spring, and significantly more in the southern and northern parts of the strait than in the middle. Considering our results along with these previous findings, there is a possibility that bottlenose dolphins are localized in the southern or northern parts of the strait during the daytime, but that some of them come to or pass through the middle of the strait during the nighttime in spring.

It is known that the behavior of small cetaceans strongly depends on the presence of prey patches (Benoit-Bird and Au, 2003). In spring, pelagic fish such as sprat (*Sprattus sprattus*), horse mackerel (*Trachurus trachurus*), and sand smelt (*Atherina boyeri*) migrate from the Marmara Sea to the Black Sea through the Istanbul Strait. It is thought that small cetaceans appear in the strait along with the fish migration. The evidence of the frequent occurrence of both families in spring is consistent with these observations. However, even in the same area and in the same season, the harbor porpoises probably present more specific location-dependent foraging behavior than the delphinids in very limited area of the middle of the strait. Tonay et al., (2007) reported that the sprat is one of the primary prey species for the harbor porpoise, particularly in spring and early summer. This migrating pelagic fish tends to form large schools. Although research on the stomach contents of delphinids in the Black Sea and the Istanbul Strait is limited, Birkun (2002) suggested that delphinids primarily prey on whiting, mullets, turbot, and thornback rays, which do not migrate between the Marmara Sea and the Black Sea in spring. By taking these previous studies into account, I can conclude that the harbor porpoises were probably foraging for migrating pelagic fish, mainly sprats, in the limited area in the south of the monitoring station in spring. On the other hand, delphinids appeared in the middle of the straight, migrating back and forth between the north and south part during the nighttime.

Major migrations of small pelagic fish in the strait take place mainly in spring. This could be the reason for the behavioral convergence between spring and other seasons in harbor porpoises. Except for spring, harbor porpoises searched not only over short ranges but also over long ranges and were present in all directions, which was clearly different from their occurrence pattern in spring. Delphinids seem to maintain a consistent behavioral pattern; no localization, nocturnal presence, and longer ICIs in autumn and winter. The delphinids retained their behavioral pattern from spring into

summer. This also supports our hypothesis that delphinids did not focus on small migratory prey in the middle of the Istanbul Strait.

4.4.2. Conclusion

Dietary selection was suggested as one of the main reasons for the different habitat use in the middle of the strait by the two families at the same time. This may be one reason why aggressive behavior between sympatric small cetacean species has not been observed here. They mainly separate by time of occurrence between seasons except for spring and summer, and separate by their utilization in seasons when they coexist, probably because of food preferences. The habitat separation between sympatric species observed in this study occurred in a quite small area (a few hundred meters) for months. It suggests that fine-scale monitoring is necessary to understand the niche partitioning. The importance of fine-scale monitoring of habitat partitioning between harbor porpoises and bottlenose dolphins was also suggested in Moray Firth, where temporal habitat separation was observed within a small area (Thompson et al., 2004). Acoustic monitoring enabled us to observe underwater sympatric ecology with higher spatial and temporal resolution than previous studies.

Chapter 5

Distribution and Density

5.1. Introduction

Information on the distribution and population density is essential for the conservation management of wild animals. Most of this information has been obtained based on visual observation; however, the observation of submerged animals, such as small cetaceans, poses a challenge. Recent rapid development of the PAM method has enabled the estimation of the distribution and population of submerged animals by monitoring of their sounds (Marques et al., 2013; Thompson et al., 2015).

The Istanbul Strait is one of the narrowest straits in the world and is 31 km in length, 0.7–3.5 km wide, and 35.8–110 m in depth with an average of 36 m (Alpar and Yüce, 1998). It is known that two families are regularly observed, consisting of three species of small cetaceans (Öztürk and Öztürk, 1996), namely harbor porpoises, bottlenose dolphins, and short-beaked common dolphins. The harbor porpoise population observed in the Black Sea is considered as endangered. In addition, both delphinids belong to the Black Sea subpopulations, which are considered as threatened (Birkun 2008; 2012; Birkun et al., 2008). Early studies suggested that small cetaceans migrate from the Marmara Sea to the Black Sea through the strait; however, heavy marine traffic and ecological stress, such as underwater noise or pollution, might at present be preventing their regular migration (Öztürk and Öztürk, 1996). Currently, there is only a single published study regarding the distribution of bottlenose dolphins in the strait (Baş et al., 2014). Baş et al., (2014) suggested that the core distribution area of

bottlenose dolphins is at the north entrance of the Black Sea and south entrance of the Marmara Sea during spring to summer, and localized only at the south entrance of the Marmara Sea during autumn to winter. However, there is currently no available information regarding the distribution of other species for the area.

Recent development of the PAM methodology has enabled us to determine the habitat (Cañadas and Hammond, 2008; Yack et al., 2013), estimate absolute abundance (Kyhn et al., 2012; Marques et al., 2013), or identify species (Kameyama et al., 2014; Lin and Chou, 2015).

In the present study, a towed PAM was conducted to reveal the distribution and density of harbor porpoise and delphinids. As mentioned in Chapter 2 and Chapter 3, the estimation of density in the area using acoustics has posed a challenge as several species are observed simultaneously, resulting in false discrimination problems. In addition, counting of individual sounds in this strait has been difficult because of heavy shipping noise and the observed species tend to make group. Therefore, I use the newly proposed density estimator detailed in Chapter 3 to estimate density, which can cover a wider spatial area based on acoustic cue counting, with accounting for the proportion of false positive or true negative discrimination errors.

5.2. Materials and Methods

5.2.1. Study Area

I conducted boat based towing survey in the Istanbul Strait, Turkey (Fig. 1.3) Acoustic observation and visual observation were conducted simultaneously in 2 or 3 days of 3 month in February, April, July 2013. Only the visual observation was conducted in October 2013. I took a round trip between south of the strait, Üsküdar (41°01′403″N, 29°00′557″E), and entrance of the Black Sea, Poyraz (41°12′471″N, 29°13′358″E) which is approximately 50 km long. For data analysis, I used the data obtained either of one way. Therefore, the survey length is around 25 km as summarized in Table 5.1.

5.2.2. Acoustic Observation

The towing type of acoustic data-logger, A-tag (ML200-ASII; Marine Micro Technology, Inc., Saitama, Japan) was used as acoustic detector. A-tag is an event data-logger, which records sound pressure and the time differences in sound arrival (TDOA) between 2 hydrophones, when the sound exceeds a preset threshold (138 dB peak-to-peak re 1 µPa). It does not record the waveform itself. Each hydrophone was sensitive at a different frequency. The first and second ones were most sensitive at 130 kHz and 70 kHz, respectively (Fig. 2.1) which was placed 189.0 mm apart. Two A-tags were towed from the stern of the boat using the 70 m length of rope. A-tags were fixed at 45 m and 65 m behind the boat in July 2013, and 25 m and 45 m in other surveys to keep the distance between two A-tags constant as 20 m. I used the front A-tag only to calculate the distance between the detected click trains to the back A-tag. During towing survey, survey boat was operated at a speed of 1.6–2.0 m/s upstream and 2.9–3.2 m/s downstream on average. This is faster than average swimming speed and close to assumed upper limit of speed of animals (Akamatsu et al., 2007; Westgate et al., 1995). Therefore, animals were not double counted acoustically because they always pass from bow side to stern side. In addition, I kept looking at the heading direction and behavior of animals in case that they swim faster than boat, but it did not happen.

The maximum detection range for each family was estimated using the following parameters: maximum source sound pressure level (Source SPL), which was estimated at 227 dB peak-to-peak re 1 µPa for bottlenose dolphins (Simard et al., 2010) and 205 dB peak-to-peak re 1 µPa for harbor porpoise (Villadsgaard et al., 2007); the minimum received sound pressure level of the present A-tag (Received SPL: 138 dB peak-to-peak re 1 µPa); and the absorption coefficient of the Istanbul Strait (α). Assuming average salinity of upper layer was 17.5, temperature was 15 degrees Celsius, α was estimated at 0.026 dB m^{-1} for 130 kHz and 0.013 dB m^{-1} for 70 kHz, respectively, by using the Francois and Garrison model (Francois and Garrison 1982a; Francois and Garrison 1982b). The maximum detection range d was estimated using Eq. (2.1).

The maximum detection range for harbor porpoise was calculated at 630 m, while that of bottlenose dolphin was more than 1,830 m. It is important to note that the maximum detection range is for the loudest clicks of on-axis beam. The detection

distance of off-axis beam or sounds of low source level could be much shorter than the above estimated values.

5.2.3. Visual Observation

In total, four or five observers were employed during each survey. Two observers were seated side-by-side on the top of the boat and were responsible for observing an area bounded by 90 degrees to the direction of travel, respectively. Observers and data recorders were changed at least once in every hour to avoid distraction. During the visual observation, I recorded the time, species, distance, angle from the boat, group size, and swimming direction of each sighting. However, I used only the time and species information data within the current study. When the animals were sighted again during the return trip within a 1-km radius from the first sighted point or with a similar group composition, they were assumed to be the same animals. These observations were then eliminated from further analysis to avoid double counting.

5.2.4. Off-Line Acoustic Data Filtering to Extract Click Trains

After obtained recordings, I applied an off-line filter to extract click trains from false detections, including background noise. The filtering program ran on Igor Pro 6.2.2 (WaveMetrics, Inc., Oregon, USA). The filter consisted of two steps. The first step of the filter was to extract click-like sounds by eliminating surface or bottom reflections and ship noise, and the second step was to extract click trains to eliminate the biological noise, such as that made by shrimps, as well as sonar signals of two or more animals produced simultaneously. For the first step, I eliminated the sounds recorded within 2.5 ms, which were likely reflected sounds from the surface or the bottom, by comparison with the direct path sounds. For the second step, I applied few criteria to select successive clicks as click trains because porpoises and delphinids produce clicks as a sequence of pulsed sounds (Au, 1993). A click train was defined as a separation of more than 200 ms from the previous click (Akamatsu et al., 2007). There should be a minimum number of pulses in a click train, designated here as six or more (Akamatsu et al., 2010b; Kimura et al., 2010). The coefficient of variance (CV) of inter-click interval (ICI) was used as criteria because ICI within a click train should

change smoothly.

According to Kimura et al., (2010) in which the same monitoring device, an A-tag, and the same filter algorithm was used to estimate the density of Yangtze finless porpoises, only the CVs of ICIs in a click train were highly affected with respect to the true positive rate (correct detection rate) and the false alarm rate of click train detection. Therefore, I examined this value to estimate the correct detection rate and false alarm rate for density estimation. More details regarding the CV of ICI are described later in the "Data Analysis for Density Estimation" section in this chapter.

Acoustic family discrimination was applied following Kameyama et al., (2014). The discrimination performance was examined by the correct detection rate, the false alarm rate, and F-value. Because all acoustic data after filtering are either harbor porpoises of delphinids, the sum of the correct detection rate of delphinids and the false alarm rate of harbor porpoises is 1.

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I confirmed that the data contained many shipping noises which prevent to count individual track of animals.

Table 5.1. Summary of survey.

Date			Survey period T (s)	Survey length (km)
Year	Month	Day		
2013	February	24	10327	16.0
		25	12546	22.4
		26	12845	24.1
	April	15	13884	23.8
		18	16952	28.3
	July	5	13140	25.2
		6	6300	24.6
		8	15120	27.0
	October	3	4867	21.9

5.2.5. Data Analysis to Estimate Density

A) Proposed Density Estimator

The cue counting line transect approach with consideration of false discrimination is proposed as Eq. (5.1), which is identical with Eq. (3.8). The object of interest *n* is the detected number of click trains over a time period *T* (seconds). The false alarm rate and the correct detection rate of detections were considered as $\hat{f}_1$ and $\hat{c}_1$, respectively, in the same way as the existing method (Kimura et al., 2010; Marques et al., 2009). These were estimated based on the parameter of the off-line acoustic data processing filter as explained below. In the same sense, the false alarm rate and the correct detection rate of discrimination were considered as $\hat{f}_2$ and $\hat{c}_2$, respectively in this estimator. These were estimated from the acoustic family discrimination method (Kameyama et al., 2014; Chapter 2). α is the group size effect that equals 1 if the detected number of click trains was proportional with that of animals; ρ is the Effective Detection Radius (EDR), which was calculated from the detection function $\hat{g}(x)$; r is the vocalization rate of click trains in unit time (seconds), which was cited from a published study (Akamatsu et al., 2007; Linnenschmidt et al., 2012; Rasmussen et al., 2012). $\hat{g}(x)$ was estimated from the distance between observer and click trains.

$$\widehat{D} = \frac{n(1-\hat{f}_1)(1-\hat{f}_2)\alpha}{\pi\hat{\rho}^2 T\hat{r}\hat{c}_1\hat{c}_2} \tag{5.1}$$

B) Calculation of the Distance

The distance between click trains to observer (the back A-tag) was calculated based on the TDOA between two hydrophones. The bearing angle of the sound source can be obtained using one A-tag; therefore, the position of the sound source can be calculated by the crossing point between each bearing angle (Fig. 5.1). By assuming that the study area comprises two dimensions because the depth of the study area (average 36 m) was sufficiently small in comparison with the detection distance, the distance between the back A-tag to sound source is the sampled distance for density estimation. The perpendicular distance R_P from the sound source to the transect line was calculated using triangulation and Eq. (5.2). Therefore, R_B was given by Eq. (5.3).

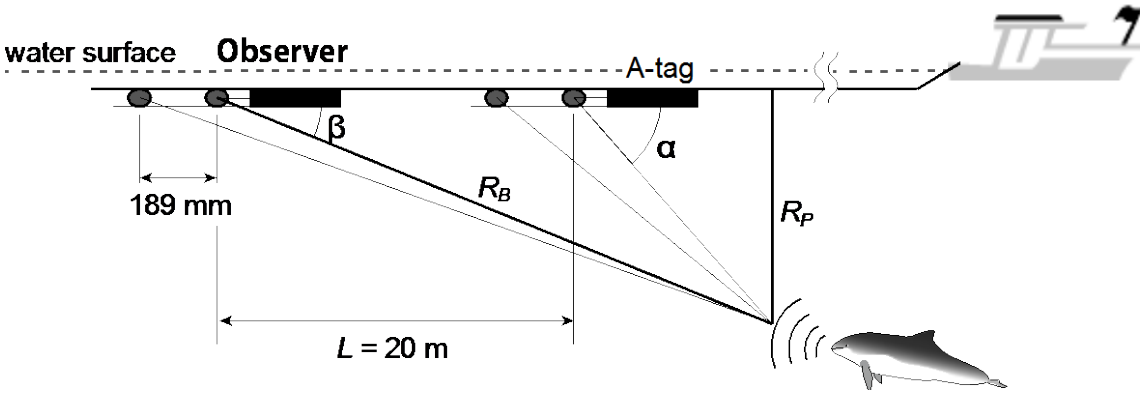

Fig. 5.1 Diagram of survey design. Two A-tags were towed behind the boat 20 m apart. The time difference of arrival of sound (TDOA) of each hydrophone equipped with a single A-tag provided the bearing angle, and the crossing point of the bearing angle enabled us to calculate the position of the sound source.

$$R_P = \frac{L * \tan\alpha \tan\beta}{\tan\alpha + \tan\beta} \tag{5.2}$$

$$R_B = \frac{R_P}{\sin\beta} = \frac{L * \tan\alpha \tan\beta}{\sin\beta\,(\tan\alpha + \tan\beta)} \tag{5.3}$$

The distance R_B was calculated click by click. Because small cetaceans emit a series of clicks as click trains, I eliminated outliers that exceeded the maximum detection range of animals and calculated the median point of all distances of clicks as the distance of the click train.

I used the analysis software DISTANCE 6.0 [provided by St. Andrews University; Thomas et al., (2010)] to estimate the detection function $\hat{g}(x)$ based on the obtained distance. Typical model functions, namely the half-normal, hazard-rate, and negative exponential distribution model, were fitted to the distance data. Model selection was conducted based on the lowest Akaike's information criterion (AIC) score. In the present study, we assume $g(0) = 1$.

C) Estimation of $\hat{f}_1$ and $\hat{c}_1$

I manually extracted all click trains detected between April 18 and July 5, 2013, in which both porpoise and delphinids were visually detected during the same day, as ground truth data of animal detection before applying a filter. The data were used as training data to correct the threshold of off-line filtering and estimate $\hat{f}_1$ and $\hat{c}_1$. Because Kimura et al., (2010) suggested that only the CV of ICI in a click train is highly affected to the correct detection or false alarm rate of detection, I changed the CV from 0.3 to 0.6 to define an appropriate detection threshold which has the highest F-value. The $\hat{f}_1$ and $\hat{c}_1$ were estimated using training data.

D) Estimation of $\hat{f}_2$ and $\hat{c}_2$

After applying the off-line filter to detect click trains with a defined threshold during the estimation of $\hat{f}_1$ and $\hat{c}_1$, I applied family discrimination following the guidelines stated by Chapter 2 and Kameyama et al., (2014). The family discrimination was performed based on the comparison of the intensity ratios of two-band frequencies (130 and 70 kHz). Because porpoises use narrow-band high-frequency clicks and delphinids use broad-band clicks, the two-band intensity distributions show different peaks. The details of a family discrimination method were described in Chapter 2 and the study by Kameyama et al., (2014).

The ground truth acoustic data was extracted from the recordings obtained on February 26. The detected species were confirmed by the visual observation. The model distribution, which was derived from ground truth data and closest to the observed data distribution, was estimated by minimizing the differential between the observed distribution and the model distribution. The appropriate threshold to discrimination, correct detection rate, and false alarm rate were adopted from the estimated model.

E) Group Effects α and Vocalization Rate r

The group effects could not be estimated in the present study; however, Kimura et al. (2010) reported that there is a linear correlation between the average number of Yangtze finless porpoise counted over every 1 min bin and that of click trains using the same algorithm of off-line filter as the present study. Therefore, I assumed that group

effect $\alpha = 1$.

The vocalization rate r was adapted from the tagging experiments on harbor porpoise reported in a previous study (Akamatsu et al., 2007). The tagged animal is a sub-adult male harbor porpoise (BL = 120 cm), which was accidentally trapped in a Danish pound net placed in the Great Belt on June 8, 2005. Although there is no report regarding the vocalization rate of click trains for delphinids, Rasmussen et al., (2012) reported the vocalization rate of clicks in different delphinids species (white-beaked dolphin *Lagenorhynchus albirostris*), and Linnenschmidt et al., (2012) reported the vocalization rate of clicks in harbor porpoises using the same device as in the present study. I estimated the average vocalization rate of click trains from these previous studies.

F) Variance of Estimation

Variance of estimated density can be approximated using the delta method (Buckland et al., 2001; Marques et al., 2009). The false alarm rate of detections $\hat{f}_1$ and that of discriminations $\hat{f}_2$ was determined by each correct detection and discrimination rate ($\hat{c}_1$ and $\hat{c}_2$). Therefore, I assumed that the various random components were independent, except for $\hat{f}_1$ and $\hat{f}_2$, and included only the variance of $\hat{c}_1$ and $\hat{c}_2$ to estimate the variance of density. The variation of r cannot be estimated from previous studies because the number of tagged animals is one; therefore, I do not include those components to estimate the variance of density as preliminary results. The variance in $\widehat{D}$ is calculated from the between variance of density and was estimated as follows:

$$\mathrm{var}\left(\widehat{D}\right) \approx \widehat{D}^2\{CV(n)^2 + CV(\hat{c}_1)^2 + CV(\hat{c}_2)^2 + CV(\hat{\rho})^2\} \qquad (5.4)$$

5.2.6. Data Analysis to Estimate Distribution

I used both acoustic and visual encounters to estimate the core area of distribution. After applying an off-line filter to acoustic data as described above, all data were checked manually to exclude heavy ship noise, which was not excluded by auto filtering. Therefore, in this case, I did not need to consider false alarm detection. The click trains detected within 10 min of each other were defined as the same encounter.

However, if the bearing angle of detections changed from the stern side to bow side within 10 min, I separated the encounter because individual animals always pass from the bow side to stern side. The 10-min duration corresponds to the approximate distance the survey boat proceeds to the maximum detection range of animals. If the acoustic and visual encounter overlapped within the same 10-min bin, only the visual encounters were used. The survey track and the geographical points of visual or acoustic encounters were imported in the software ArcGIS 10.2.1 (Esri Inc., California, USA). Kernel density was estimated using the function in ArcToolBox. By taking into account the study conducted by Baş et al., (2014), we use the same raster grid cell as 30 m. The band width was set as 650 m by accounting for the acoustic detection range of animals. The 95% and 50% core zones of distribution were calculated.

5.3. Results

5.3.1. Density

In total, 16 click trains and 66 click trains were extracted with the distance from harbor porpoises and delphinids, respectively. Among the click trains, 7 out of 16 click trains and 45 out of 66 click trains were detected during a one-way trip (one-effort) for harbor porpoises and delphinids, respectively. Therefore, $n = 7$ and 45 for harbor porpoises and delphinids, respectively. The data obtained off-effort were used only to estimate the detection function $\hat{g}(x)$. Total survey periods T was 105,981 s (approximately 29 h).

A) Estimation of $\hat{f}_1$ and $\hat{c}_1$

To estimate $\hat{f}_1$ and $\hat{c}_1$, I manually selected 145 click trains as ground truth data. According to the change of CV of ICI, the correct detection rate and false alarm rate were calculated (Table 5.2). The highest F-value (0.681) was reported when the CV of ICI was 0.45 (Fig. 5.2; 5.3) with 0.648 of the true positive rate and 0.020 of the false positive rate. The correct detection rate $\hat{c}_1$ and false alarm rate $\hat{f}_1$ were calculated as 0.648 (CV = 0.066) and 0.273 (CV = 0.000), respectively by Eq. 5.5 and 5.6.

Table 5.2. Contingency table of classification

	Ground truth click trains	Ground truth noise
Detection as click trains	True positive	False positive
Detection as noise	False negative	True negative

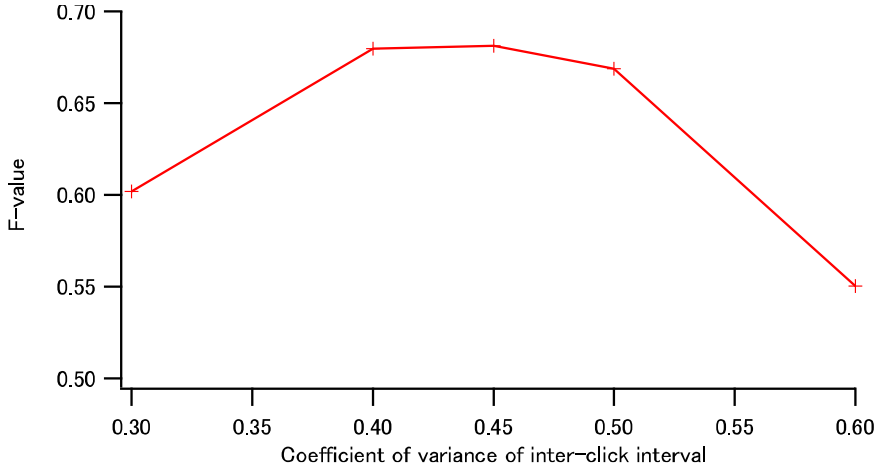

Fig. 5.2 F-value of the coefficient of variance (CV) of inter-click interval (ICI). F-value was highest as 0.648 when the CV of ICI was 0.45.

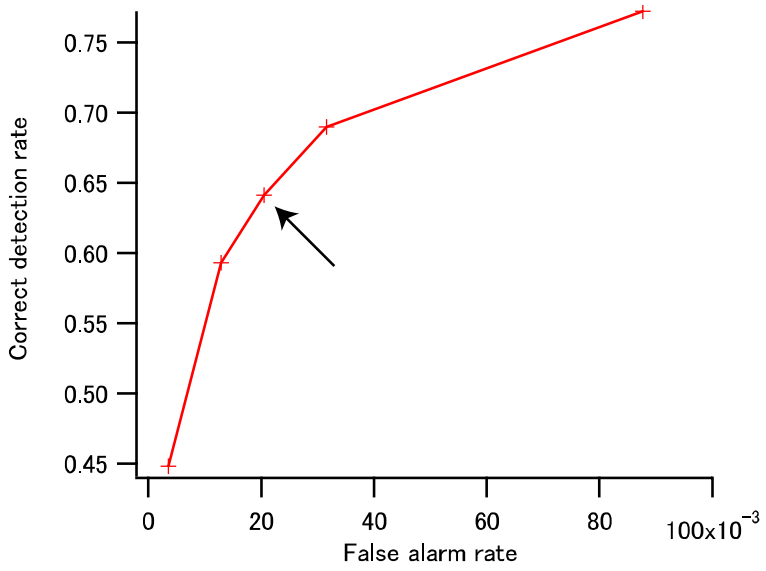

Fig. 5.3 Receiver operating characteristic (ROC) curve for change of coefficient of variance (CV) of inter-click interval (ICI). The true positive rate was 0.648 and false positive rate was 0.020 when I adopted 0.45 as the CV (indicated by black arrow).

$$\hat{c}_1 = True\ positive\ rate = \frac{True\ positive}{True\ positive + False\ negative} \quad (5.5)$$

$$\hat{f}_1 = False\ alarm\ rate = \frac{False\ positive}{True\ positive + False\ positive} \quad (5.6)$$

B) Estimation of $\hat{f}_2$ and $\hat{c}_2$

To estimate $\hat{f}_2$ and $\hat{c}_2$, I manually extracted 130 clicks from harbor porpoise and 104 clicks from delphinids, with visual species confirmation as ground truth data. It is confirmed that the two-band intensity distribution (130 kHz and 70 kHz) have different peaks in ground truth data (Fig. 5.4). Based on this ground truth data, the false discrimination rate $\hat{f}_2$ and correct discrimination rate $\hat{c}_2$ were estimated following the method in Chapter 2. Family discrimination was performed on a daily basis. The results are summarized in Table 5.3. Because no click trains with a distance were observed on February 24 and 25, these two days were not included in Table 5.3. The average of the correct discrimination rate $\hat{c}_2$ for harbor porpoise was 0.869 (CV = 0.055) and that for delphinids was 0.846 (CV = 0.035). The average of false discrimination rate $\hat{f}_2$ for harbor porpoise was 0.213 (CV = 0.501) and that for delphinids was 0.088 (CV = 0.337).

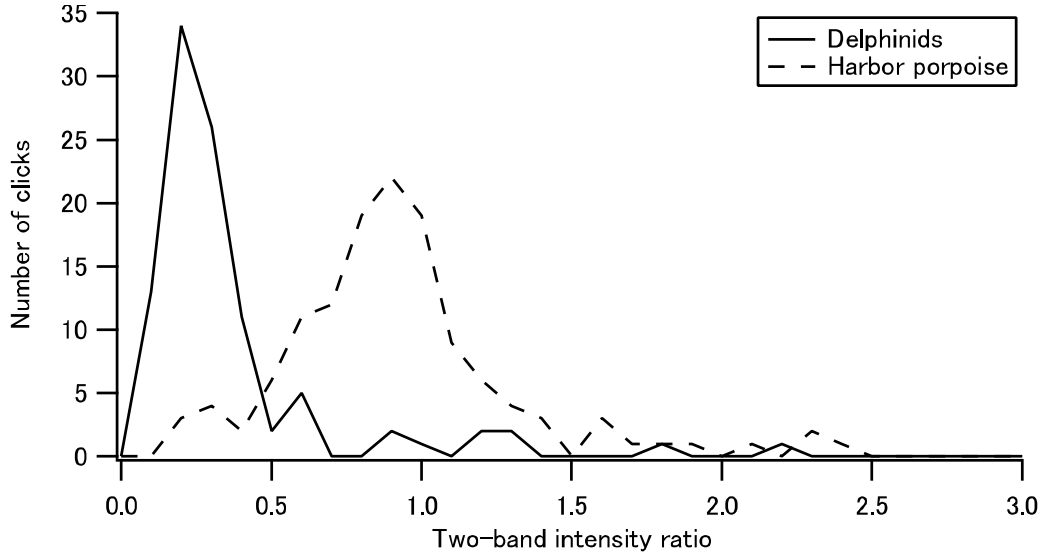

Fig. 5.4 Ground truth distribution of two-band intensity ratio.

Table 5.3. Summary of correct discrimination rate and false discrimination rate for harbor porpoise and delphinids. No click trains with distance were observed in February 24 and 25, these two days

Date				Harbor porpoise		Delphinids	
Month	Day	Threshold	Estimated % of porpoise	Correct rate	False rate	Correct rate	False rate
February	26	0.51	66	0.931	0.096	0.808	0.143
April	15	0.66	23	0.838	0.350	0.865	0.053
	18	0.66	33	0.838	0.246	0.865	0.084
July	5	0.66	34	0.838	0.238	0.865	0.088
	6	0.51	72	0.931	0.074	0.808	0.181
	8	0.66	30	0.838	0.273	0.865	0.074
October	3	0.51	53	0.931	0.155	0.808	0.088

C) Vocalization Rate r

Akamatsu et al., (2007) reported that harbor porpoises emit click trains every 12.3 s. Therefore, I used the vocalization rate $r = 0.081$ s^{-1} for harbor porpoise. As I mentioned in the Materials and Methods, there are no reports regarding the vocalization (click train) rate of bottlenose dolphins or short-beaked common dolphins. However, Rasmussen et al. (2012) reported the click rate of delphinids and white-beaked dolphins as 500 and 550 min^{-1}, respectively, and Linnenschmidt et al. (2012) reported the click rate of three individuals of harbor porpoise as 109, 108, and 403 min^{-1}. The average click rate of white-beaked dolphin (525 min^{-1}) was 2.5 times higher than that of harbor porpoise (207 min^{-1}) from those previous studies. I assume that the number of clicks in a click train and click train intervals are similar between them and used 0.203 s^{-1}, which is 2.5 times higher than the vocalization rate of harbor porpoises, as vocalization rate r of delphinids.

D) Estimation of Detection Function $\hat{g}(x)$ and Effective Detection Radius $\hat{\rho}$

Half-normal, hazard rate, and negative exponential functions were fitted as key functions on distance data for harbor porpoise and delphinids, respectively. Based on

AIC, negative exponential and hazard rate were selected for harbor porpoise and delphinids, respectively (Table 5.4; Fig. 5.5 and 5.6). The EDR $\hat{\rho}$ was estimated as 138.39 (CV = 0.22) and 128.65 m (CV = 0.27).

Table 5.4. Model selection based on AIC among typical three key functions implemented in DISTANCE 6.0. The model of negative exponential and hazard rate were selected for harbor porpoises and delphinids, respectively.

	Key function	AIC	ΔAIC
Harbor porpoise	Half-normal	187.57	1.63
	Hazard rate	186.69	0.75
	Negative exponential	185.94	0.00
Delphinids	Half-normal	352.03	70.90
	Hazard rate	281.13	0.00
	Negative exponential	302.08	20.95

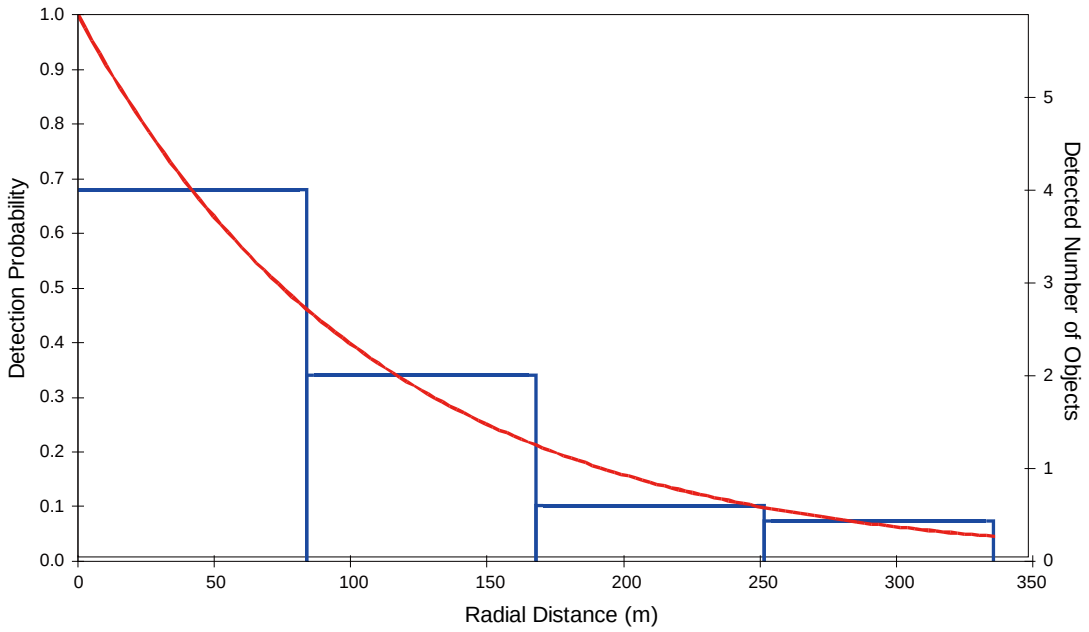

Fig. 5.5 The estimated detection function of harbor porpoise fitted by negative exponential as a key function (red line) and frequency distribution of radial distance in meters (blue bar; n = 16).

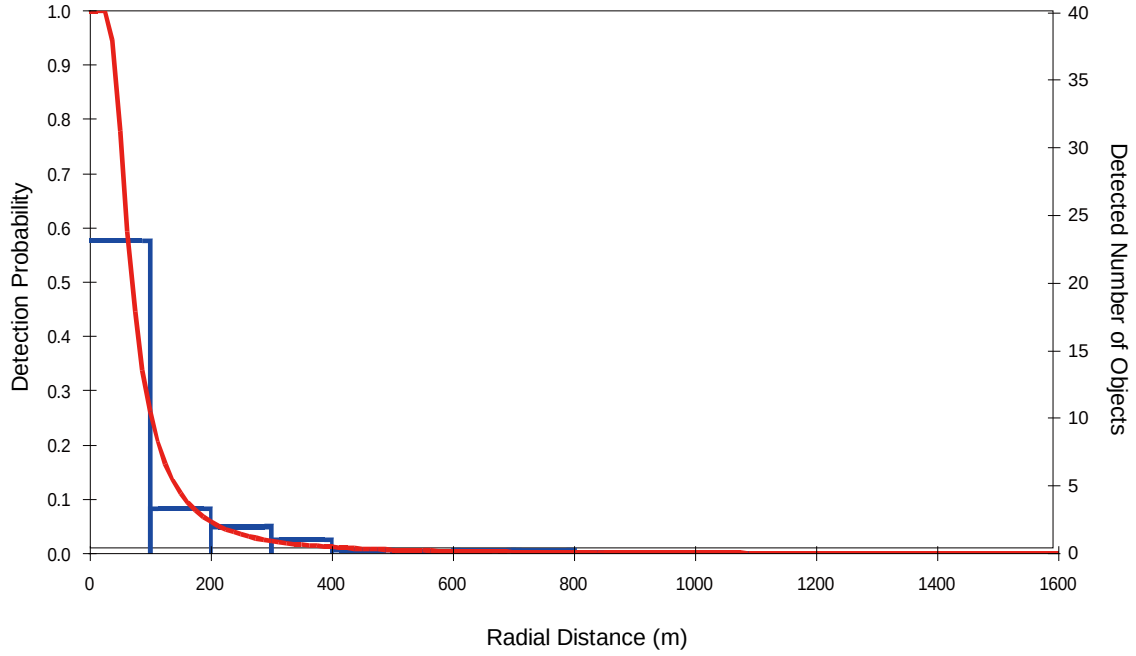

Fig. 5.6 The estimated detection function of delphinids fitted by hazard rate as a key function (red line) and frequency distribution of radial distance in meters (blue bar; n = 66).

E) Estimation of Density and Variance

All parameters for harbor porpoise were as follows: n =7 (CV = 0.564), $\alpha = 1$, $\hat{f}_1$ = 0.273, $\hat{c}_1$ = 0.648 (CV = 0.066), $\hat{f}_2$ = 0.213 (CV = 0.501), $\hat{c}_2$ = 0.869 (CV = 0.055), $\hat{\rho}$ =138.39 (CV = 0.220), $\hat{r}$ = 0.081, and $T = 105981$.

All parameters for harbor porpoise were as follows: n = 45 (CV = 0.773), $\alpha = 1$, $\hat{f}_1 =$ 0.273, $\hat{c}_1$ = 0.648 (CV = 0.066), $\hat{f}_2$ = 0.088 (CV = 0.337), $\hat{c}_2$ = 0.846 (CV = 0.035), $\hat{\rho}$ = 128.65 (CV = 0.270), $\hat{r} = 0.203$, and T =105981.

By using Equation (5.1), the density of harbor porpoise was estimated as 0.013 individuals km^{-2} and that of delphinids was estimated as 0.033 individuals km^{-2}. Variance was calculated by using Equation 5.4 as 6.67×10^{-5} for harbor porpoises and 7.49×10^{-4} for delphinids, corresponding to a CV = 0.612 (SD = 0.008) for porpoises and CV = 0.823 (SD = 0.027) for delphinids.

5.3.2. Distribution

The geographical point of encounters of harbor porpoise (n = 14) and delphinids (n = 21) were depicted with 95% and 50% kernel density, respectively (Fig. 5.7 and 5.8). The average encounter rates throughout the season were 0.54 km^{-1} for harbor porpoises and 0.81 km^{-1} for delphinids. The 50% core area of the harbor porpoises were estimated as the middle to north of the strait; however, their distribution appeared to disperse, and they were observed over the entire strait. The encountered number was six during February, three during April, and five during July. The 50% core area of delphinids was estimated in the north of the strait. Both harbor porpoises and delphinids tended to be encountered at a similar point regardless of seasons, particularly in delphinids. Average group size was obtained by visual observations and 4.09 for harbor porpoise, 12 for bottlenose dolphin and 6.25 for short-beaked common dolphin. The group size was not considered in the distributions.

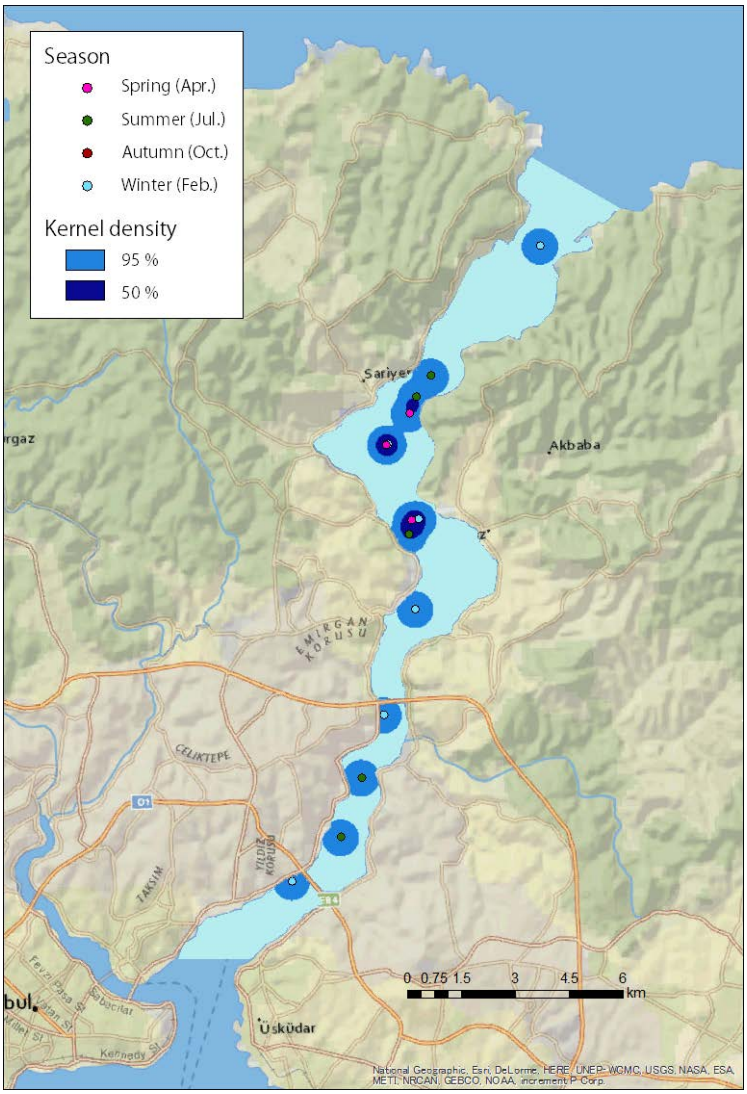

Fig. 5.7 Kernel density and seasonal distribution of harbor porpoises (n = 14).

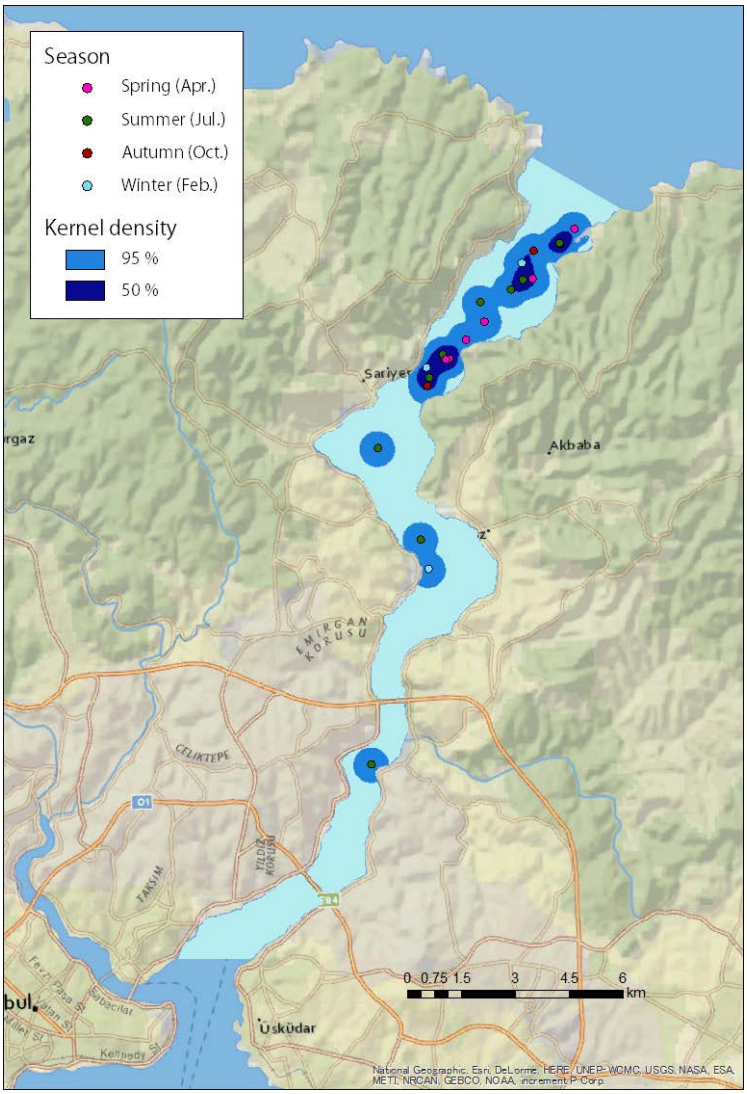

Fig. 5.8 Kernel density and seasonal distribution of delphinids (n = 21).

5.4. Discussion

5.4.1. Density

A) Advantages and Disadvantages of the Presented Density Estimation Design

In the present study, I presented a novel distance sampling survey approach to estimate density from acoustic cues with consideration of misclassification of species, which combines the line transect survey design with a point transect estimation. The standard method of a line transect uses the distance between survey lines to individual animals (Marques et al., 2013). However, the counting of individual animals in the density estimation using PAM is difficult because of biological or anthropogenic underwater noises and for the species that tend to make big group. Although there are some studies that counted individual animals using an acoustic towing array, such as for the sperm whale (Hastie et al., 2003; Lewis et al., 2007) and vaquita (Gerrodette et al., 2011), these studies were conducted in a relatively high signal-to-noise level. In addition, recognizing individuals become more difficult if animals form groups. On the other hands, an acoustic cue-based point transect has been used to estimate the density of many species, such as the finless porpoise (Kimura et al., 2010), beaked whales (Marques et al., 2009), right whales (Marques et al., 2011), minke whales (Martin et al., 2013), and fin whales (Harris et al., 2013). By using this method, I do not need to count individual animals; however, a sufficient number of monitoring points are necessary to extrapolate the density of large survey areas. The proposed cue counting line transect survey design solves these drawbacks of previous studies because it enables the use of acoustic cues instead of individual animals and cover a large survey area with a minimum of two acoustic detectors.

Another advantage of the presented estimation method is that this method explicitly takes into account the probability of false discrimination of species. Acoustic discrimination of small cetacean species remains a challenging issue for PAM; however, the recent development of this methodology made it possible for the acoustic discrimination of some species and areas (Kameyama et al., 2014; Lin and Chou, 2015; Oswald et al., 2007; Roch et al., 2007). This progress enables us to estimate density in consideration of the accuracy of species discrimination. The misclassification of species

has become a common issue in density estimation not only for PAM but also using drones. Johnston et al., (2015) noted that 95% of individuals could be identified at the species level in boat-based visual observation, whereas only 23% of individuals could be identified using aerial digital imagery visual observation in a seabird survey. Conn et al., (2014) and Johnston et al., (2015) estimated the population density considering the false discrimination error for ice-associated seals and sea birds using drone-based visual observation, respectively. However, until very recently as the study by Johnston et al., (2015) stated, there are not many described studies which account for the accuracy of species classification in estimates of abundance. Moreover, to date, there are no explicit methodologies to consider false discrimination using PAM.

The disadvantage of the presented estimation method is that as more parameters were added, variance was increased. Caillat et al., (2013) simulated how false discrimination affects the variance of estimated density and revealed that it becomes quite large if the correct classification rate was very low (less than 0.52 in that study) and/or species difference in the encounter rate was very large (less than 10% of all detections) among four species calls. In the case of our data, the false alarm rate and correct classification rate of two species was estimated by using the sound intensity of a two-band frequency band as described in Chapter 2 and Kameyama et al., (2014). The results showed that the correct detection rate was between 0.81 and 0.93 and that the lowest encounter rates of the sounds of each family were 23% for harbor porpoise and 28% for delphinids (Table 5.3). Therefore, the effect of misclassification to final density is relatively small.

The effect of vocalization rate is quite large on density estimations for all acoustic cue-based estimations, including our presented method. As a previous study indicated, to obtain a reliable vocalization rate remains difficult, mainly because of the low sample size of the reported number of individuals (Evans and Hammond, 2004; Martin et al., 2013). In the case of our study, the vocalization rate of harbor porpoise was obtained from one individual and that of delphinids was extrapolated from white-beaked dolphins. If I could obtain a sufficient sample size of vocalization rate from target species, and ideally in the survey area, the reliability of density becomes higher. Further studies of a bio-logging type of survey, which enables us to estimate

vocalization directly, may reduce this uncertainty of vocalization rate.

B) Animal Density in the Istanbul Strait

This is the first report of animal density of small cetacean species in the Istanbul Strait. I could not divide the two delphinids species because of the limitation of the methodology; however, the year-round average abundance of harbor porpoise and delphinids are basic ecological information required to understand the occurrence of small cetaceans. The closest area from the Istanbul Strait in which the density was estimated was the south eastern Black Sea within Georgian territorial waters. The density of harbor porpoises, bottlenose dolphins, and short-beaked common dolphins were estimated as 1.53, 0, and 4.18 individuals km^{-2}, respectively using the boat-based visual observation survey conducted during 3 days in January 2006 (Birkun et al., 2006; IUCN 2015). There was no estimated density within Turkish territorial waters; however, the occurrence of all three species, including bottlenose dolphins, was confirmed (Gönener and Özdemir, 2012; Tonay and Öztürk, 2003). The estimated density in the present study was 0.013 individuals km^{-2} for harbor porpoise and 0.033 individuals km^{-2} for delphinids, which were relatively low for harbor porpoises and bottlenose dolphins compared with the density of the south eastern Black Sea within Georgian territorial waters. However, the low density is not unusual because the Istanbul Strait might be a migrating corridor for small cetaceans that are subspecies belonging to either the Black Sea or the Marmara and Aegean Sea subpopulations (Öztürk and Öztürk, 1996). The animal density in the other corridor for the Black Sea subpopulations, namely the Kerch Strait between the Black Sea and Sea of Azov, was estimated as 0.063 individuals km^{-2} for harbor porpoise and 0.147 individuals km^{-2} for bottlenose dolphins using the boat-based visual observation survey conducted during 6 days in August 2004 (Birkun et al., 2004; IUCN 2015). There is almost no record of short-beaked common dolphin in the Kerch Strait.

The estimated density was higher for delphinids than for harbor porpoises. Although I cannot conclude that delphinids appear in the strait more frequently than harbor porpoises because of the high CV value, this result is consistent with visual observation in the strait, indicating that the number of detected individuals was most

abundant for bottlenose dolphin, followed by short-beaked common dolphins and harbor porpoises (Öztürk et al., 2009).

5.4.2. Distribution

The year-round distribution of small cetaceans was investigated only for bottlenose dolphins based on theodolite visual observation (Baş et al., 2014). Baş et al. (2014) revealed that bottlenose dolphins localized the north and south entrance of the strait as a 50% kernel core area during spring and summer and only the south entrance of the strait during autumn and winter. In our study, it was consistent that the 50% kernel core areas of delphinids were observed near the north entrance of the strait (Fig. 5.8). In addition, 16 out of 21 encounters of delphinids were detected during spring and summer, and 13 of them were detected in the nearby north core area in the strait. In our survey, I considered that the south entrance of the strait belongs to the Marmara Sea rather than the strait; therefore, I did not cover the south core area estimated by Baş et al. (2014). There are no published studies regarding the distribution of harbor porpoises in the strait. The observations were relatively dispersed compared with those of delphinids. The 50% kernel core area were estimated south of the strait (close to the center part) compared with that of delphinids. The 50% kernel core area did not overlap between delphinids and harbor porpoises.

5.4.3. Conclusion

This is the first report of animal density of harbor porpoises and delphinids and the distribution of harbor porpoise in the Istanbul Strait. The low density of both families made obtaining a sufficient sample size difficult. However, these results implied that the total abundance and distribution was different between families within the strait. Long-term observation is required to further understand behavior characteristics, including seasonal change of density and the distribution in each family

Chapter 6

General Discussion

6.1. Ecology of the Small Cetaceans in the Istanbul Strait with the Implication for Conservation

In this study, I proposed acoustic species discrimination method (Chapter 2) and density estimation method (Chapter 3) which complement the defects of existing PAM methodology. In addition, I applied proposed methods to reveal the seasonal change in presence and underwater behavior of harbor porpoise and delphinids (Chapter 4), and to estimate the density and distribution (Chapter 5) in the Istanbul Strait. The possible difference of strait utilization between harbor porpoise and delphinids was revealed by summarizing the results in Chapter 4 and Chapter 5.

The harbor porpoises were observed within whole strait, but dispersed core areas were estimated in the middle to north parts of the strait from the towed PAM. Two-years fixed PAM in the middle point of the strait revealed porpoises were mainly detected from spring to summer and almost no detections in winter. Previous study suggested that the small cetaceans come into the strait affected by the migrating pelagic fish (Öztürk and Öztürk 1996) which is suggested as the main prey in spring to early summer for them (Tonay et al., 2007). Our results were consisted with these studies, porpoise appeared to the monitoring area in spring to summer, and have used the sonar signals with short ICIs in spring. It is known that the short ICIs were observed when they were either of socializing or feeding. Taking into account the previous studies, the observed short ICIs possibly connected with the feeding behavior. Besides prey

availability, marine traffic may affect the presence on animals; however, the all commercial fishing were banned regardless of seasons around fixed monitoring area and the number and type of vessels observed around monitoring area is not highly variable among seasons (Baş et al., unpublished data; Dede et al., 2013). Therefore, ship traffic is unlikely to provide the reason of seasonal difference in presence.

The delphinids were mainly observed in the north part of the strait by the towed PAM. This localization trend was supported also by previous study (Baş et al., 2014). Previous visual observation suggested that there are not many encounters in the middle point of the strait without summer; however, two-years fixed PAM in this area revealed that they were detected during nighttime throughout the seasons. The results of our studies did not show clear seasonal change of occurrence frequency and sensing distance, it did not support the hypothesis that small cetaceans come to the strait for foraging in spring at least in the middle of the strait. It is suggested that the primary pray species are slightly different between harbor porpoise and delphinids (especially bottlenose dolphin) in the Black Sea (Birkun 2002). In addition, bottlenose dolphin distribute in the south and north part of the strait during daytime in spring (Baş et al., 2014). Taking into account these previous studies, it is suggested that the delphinids distribute north or south of the strait during daytime but come to or pass through the middle of the strait during the nighttime, and used different prey or geographical prey ground from harbor porpoise.

This study revealed the possible separation of geological distribution and feeding ground/species between harbor porpoise and delphinids within the Istanbul Strait. In terms of conservative ecology, we need to set the different conservation management strategy for each species based on the difference of their usage of the strait. Our results suggest that small cetacean species, especially delphinid species, appear in the middle of the strait not only in spring but also in other seasons. Baş et al., (2014) proposed MPAs for bottlenose dolphins with seasonal priority, including the middle of the strait during spring. I agree that the middle of the strait is important to both harbor porpoises and delphinids as a feeding ground and traveling pathway in spring; however, we should consider that delphinids might use this area throughout the year during the nighttime. This mismatch is a good example of showing the traditional visual

observation might not enough to construct effective management strategy. It is necessary to use multiple methods, such as PAM for nighttime observation, tracking by tag to estimate individual home range or genetic sampling to examine the group structures, to understand the real biology of animals.

6.2. Applicability of the Proposed Methods to Other Species

In this study, I proposed acoustic species discrimination method (Chapter 2) and density estimation method combining the line transect approach to the point transect estimation. I discuss the possibilities of future applications of proposed method in this section.

The acoustic discrimination between harbor porpoise and delphinids with estimation of accuracy is possible by using one parameter, two-frequency intensity comparison, in proposed method. I developed this method using the acoustic event data-logger A-tag in the Istanbul Strait; however, this method can be applied to other species producing narrow-band high frequency (NBHF) and broad band clicks, and by other acoustic device. So far, totally three families, one genus and two species were reported as producing NBHF clicks; family Kogiidae, Phocoenidae, Pontoporiidae, genus *Cephalorhynchus*, and two species of *Lagenorhynchus* hourglass Dolphin and peale's dolphin (Morisaka, 2012). Whereas, most of other small cetaceans produce broad band clicks. There are many areas in the world that both NBHF clicks type and broad band clicks type species are observed simultaneously. Especially most of family Phocoenidae species inhabit coastal water, which is the area many PAMs are conducted in, without overlapping the habitat with more than two NBHF clicks type of species. The proposed method is effective to observe coastal NBHF clicks type of species with discrimination from other broad band type of species. In addition, two-band intensity ratio between low frequency (around 70 kHz) and high frequency (around 130 kHz) can be obtained by other broad band acoustic recorders. Obtaining the ground truth acoustic data which is confirmed by visual observation is the most important part of this method. If we obtain the ground truth data once, the proposed method can be used for different species independent from the monitoring device.

The density estimation method which combine the line transect approach with

point transect estimation by considering misclassification of species was proposed in present study. The previous acoustic line transect sampling need to count the individual animals to estimate density, by counting the number of track line of sounds as individual (Gerrodette et al., 2011), the number of each detection as individual by assuming that animals are not to be detected as cluster (Gillespie et al., 2005; Hastie et al., 2003) or the number of each detections with estimation of the group size obtained by visual observations (Barlow and Taylor 2005). If the survey area was exposed by high density of marine traffic, sometimes the track of phonating animals were covered by underwater noises. These heavy marine noises make the individual counting difficult. In addition, it is not reasonable to assume that animals were detected not as cluster except some solitary species. On the other hands, point transect sampling do not need to count individual animals, we need the acoustic cue instead. However, we need several number of monitoring device to cover large survey area, it is costly and might need some effort to equal the parameter of a detections among different devices. Present method is effective in the area that has similar situations as the Istanbul Strait which is exposed heavy marine traffic and difficult to employ several monitoring device.

Similar with the visual observations, PAM is now one of the standard methods to study marine mammals. However, still there are some limitations to expand the applicable study area and species, such as ambiguity of species classifications and deficiency of standardized equipment or detection threshold. I believe this study solved some of these problems and contributed for further development of PAM methodology.

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