# Doctoral Thesis

# Development and application of a fine-scale positioning method for the observation of movement behaviour of fish schools

Supervisor     Associate professor Hiromichi MITAMURA

Department of Social Informatics
Graduate School of Informatics
Kyoto University

Junichi TAKAGI

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## Abstract

Collective movement provides various advantages to animals; enhanced navigational ability is one of these advantages. In the absence of a leader, individuals in a group choose movement directions by compromising with other members, and this leads them to follow a more accurate route. Theoretically, it is the 'many-wrongs principle' that allows groups to have improved navigational ability, in which a more accurate compass is yielded simply by pooling information (in the group) from many inaccurate sources of directional information (individuals). Collective navigation has been studied empirically in many terrestrial animals, while relatively few studies have focused on this aspect in aquatic animals, including fish, due to technological limitations and the wider ranging migrations undertaken by such animals. However, a new biotelemetry system using novel technology has recently been developed; this technology theoretically assesses collective navigation by schooling fish in the field. This system was applied herein to the black rockfish, *Sebastes cheni*, which occurs in coastal rocky areas around Japan. This species is well-known for having a small home range and the ability to navigate back to its original habitat after displacement. In the general conceptual framework of movement ecology, external factors, including the presence of conspecifics, influence the movements of individuals. If black rockfish recognize other conspecifics, their movements could be motivated by the presence of other individuals. There is the possibility that black rockfish exhibit collective navigation in their homing journeys. Based on this, I developed the following two hypotheses: (1) black rockfish would return to their original location after artificial displacement while forming a group; and (2) group homing would improve the efficiency of homing behaviour compared with homing by a single fish because of the benefits of collective navigation.

The objectives of this research were as follows: to develop a fine-scale positioning method using the new biotelemetry system that can observe schooling behaviour, and subsequently to test the two hypotheses proposed by applying this newly developed positioning method in the field.

In Chapter 2, prior to developing a positioning method, the ability of the

biotelemetry system to simultaneously identify individual entities while avoiding multi-path effects was evaluated in the field. Eight transmitters with a 1.28 s signal transmitting interval were deployed at the same location. The detection rate during 15 min of recording was ca. 75.0%. The results showed that multiple signals from several transmitters were simultaneously identified at a high temporal resolution (<2 s). Depth data encoded in signals provided accurate (<0.4 m) and correct (>84%) information. The biotelemetry system thus had adequate potential for the observation of schooling fish in the field.

In Chapter 3, the fine-scale positioning method was developed and applied to the observation of multiple fish in the field. This method pinpointed stationary transmitters with a positional precision of <10 cm and positioning interval of <10 s. As an example of the application of this method, the intermittent stop-and-go locomotion of Siebold's wrasses, *Pseudolabrus sieboldi,* was observed during their homing behaviour. Their turn angles were investigated, which suggested that the intermittent 'stop' phase of their behaviour is linked to reorientation while homing.

In Chapter 4, I tested my two hypotheses regarding the collective movement of homing black rockfish using the fine-scale positioning method developed in previous Chapters. Two groups, consisting of four individuals each, and four single individuals were released, and then their homing journeys were observed. In group-released individuals, two rockfish moved together just after release within a short period of ca. 100 s. This partially supported my hypothesis (1) concerning their collective movement. However, my other hypothesis (2) of their navigational ability was not supported.

In these studies, I developed a fine-scale spatiotemporal positioning method for fish schools utilizing novel technology and biotelemetry techniques, and applied it to testing hypotheses about the occurrence of collective navigation in fish. The hypotheses proposed were not completely proven, but were partially supported. The positioning method developed herein has great potential to contribute to providing empirical data on group behaviour in fish, including not only the schooling behaviour of conspecifics but also inter- and intra-specific interactions, such as predator-prey and male-female interactions, in the field. These data will enhance the efforts to link movement ecology the study of group behaviour and/or collective movement.

Development and application of a fine-scale positioning method for the observation of movement behaviour of fish schools

# Contents

# Chapter 1

# **Background**

## **1.1 Collective movement for navigation**

Organisms live in groups that exist in time and space at almost all conceivable scales. Living in a group itself and/or collective movement provides various advantages (Krause and Ruxton, 2002; Sumpter, 2010), such as increased food searching efficiency (Pitcher et al., 1982), reduced predation risk (Partridge, 1982; Magurran, 1990), and reduced energy expenditures during locomotion (Weihs, 1973; Marras et al., 2014; Portugal et al., 2014; Hemelrijk et al., 2015). Researchers have long studied and been fascinated by collective animal movements, such as those performed by schooling fish, flocking birds, herding ungulates, swarming insects, and other groupings of many individual animals moving together in a highly coordinated manner. Recently, technological advances and insights from theoretical models have allowed increasing amounts of empirical data on collective animal movement to be accumulated through field studies (Westley et al., 2018; Berdahl et al., 2018; Hughey et al., 2018).

Enhanced navigational ability is another advantage of collective movement (Sumpter, 2010). When individuals travel in a group, two types of navigational information are merged, specifically the experience or internal compass of each individual and the direction taken by other members in the group. In the absence of a leader, individuals in a group

choose the group's movement direction by compromising with other members in the group, and thus they are led to follow a more accurate route. For example, the homing efficiency of pigeons was improved when they travelled in flocks compared with when individuals travelled alone (Biro et al., 2006; Dell'Ariccia et al., 2008), and king penguin chicks returned to their original nesting place (créche) more efficiently when two chicks travelled together than when individuals travelled alone (Nesterova et al., 2014). Theoretically, it is the 'many-wrongs principle' that allows groups to have improved navigational ability. This intriguing principle was first proposed by Bergman and Donner while studying migrating birds (Bergman and Donner, 1964). They suggested that there was 'a smaller variation in the headings of individual flocks than in the case of individual birds,' and thus migration in flocks 'increases the accuracy of the orientation mechanism' (Bergman and Donner, 1964). The 'many-wrongs principle' is based on the simple idea that, in the context of navigation, more accurate directional information is yielded by pooling information (i.e., in the group) from many inaccurate directional information sources (i.e., individuals): thus, collective movement averages out errors derived from individuals, and the overall navigational ability of the group is improved (Simons, 2004; Codling et al., 2007).

Navigational ability has been well-studied in seabirds, such as penguins (e.g., Trathan et al., 2008) and albatrosses (e.g., Bonadonna et al., 2004), as well as in turtles (e.g., Brothers and Lohmann, 2015) and in anadromous and catadromous fishes, such as salmonids (e.g., reviewed in Ueda 2012) and eels (e.g., reviewed in Baltazar-Soares and Eizaguirre, 2017). However, the vast majority of empirical studies focusing on collective navigation have been conducted on terrestrial animals. The navigational ability of schooling fish has been studied far less, although the importance of collective behaviour in homing fish is well known. To my knowledge, one study did make and test hypotheses concerning collective navigation in salmonids (Berdahl et al., 2014). However, the present study was based on yearly population data, not on empirical movement data obtained in the

field as is done in studies of terrestrial animals.

One of the problems preventing the collective navigation of schooling fish in the field from being the target of more studies could be the fact that these animals undergo wider ranging migrations than many terrestrial animals. Salmonids and eels, for example, accomplish trans-oceanic navigations back to a natal river or a natal marine area. Such migration is too extensive for the entire movement route to be observed. Thus, to investigate collective navigation in fish based on positional information, it is necessary to consider target fish species that exhibit homing behaviour after displacement within a limited area, i.e., short-range homing.

## 1.2 Target species and behaviour

### 1.2.1 Target coastal fish species: black rockfish

There are several coastal fish species that show site fidelity and homing ability, such as wrasses (e.g., Chateau and Wantiez, 2007), groupers (e.g. Kaunda-Arara and Rose, 2004), damselfish (e.g., Booth, 2016), and cardinalfish (e.g., Marnane, 2000). The species-rich rockfish genus *Sebastes* includes over 100 species (Eschmeyer and Hureau, 1971; Kendall, 1991; Kendall, 2000), many of which are known for their strong site fidelity and homing ability (e.g., Green and Starr, 2011; Matthews, 1990a; Mitamura et al., 2002, 2005, 2009, 2012; Reynolds et al. 2010). In Japan, the black rockfish, *S. cheni* (Fig. 1-1), is one of the most common and commercially important *Sebaste*s species. This species is found along the coast of Japan, from southern Hokkaido in the north to as far southward as Kyushu, on both the Pacific and the Japan Sea coasts (Harada, 1962). Black rockfish inhabit *Zostera* and *Sargassum* beds in their early life history stages and are active in the daytime at these stages, whereas adult black rockfish inhabit rocky reefs and are active mainly at night. They feed on small fish and marine invertebrates (Harada, 1962).

*S. cheni* is sympatric with (i.e., inhabits the same area as) the congeneric species *S. inermis* and *S. ventricosus*. There are few morphological differences among these three species, so genetic analysis is necessary to distinguish them completely from each another (Kai et al., 2002; Kai and Nakabo, 2008). However, the mode number of pectoral fin rays can enable one to roughly distinguish these three species, as this is 15, 16, and 17 for *S. inermis*, *S. ventricosus*, and *S. cheni*, respectively (Kai & Nakabo 2002; Kai et al., 2002). Additionally, *S. cheni* tends to inhabit the inner coastal area more than the other two species (*S. inermis* and *S. ventricosus*) do (Kai and Nakabo, 2002). The field study in this research (Chapter 4) was conducted in a coastal area, but as it is difficult to perform genetic analysis during the field study, I could not use this approach to confirm the specific identity of the fish studied. However, I counted the number of pectoral fin ray of the specimens examined in the field, and based on this concluded that they most likely represented the target species, *S. cheni*.



**Fig. 1-1.** Target species, black rockfish *Sebastes cheni*,

## 1.2.2 Hypotheses of collective movement

Adult black rockfish, as mentioned above, inhabit rocky areas and each individual maintains a small home range (Harada, 1962; Mitamura et al. 2009). This species also has the ability to navigate back to their original habitat after an artificial displacement of 1–4 km (Mitamura et al., 2002). It is thought that they predominantly use the olfactory sense when homing from an unfamiliar area (Mitamura et al. 2005; 2012), but they may also use vision to navigate within a familiar area (Mitamura et al. 2012). Black rockfish form large schools in their early life history stages, whereas adult black rockfish are rather solitary and usually do not form groups (Harada, 1962), although they do aggregate around rocky areas that represent favourable habitats. However, there is no clear evidence whether they move in schools or not. In the general conceptual framework of movement ecology, external factors, including both biotic and abiotic environment, form one of the four components (the other three are internal state, motion capacity, and navigation capacity) that influence movement (Nathan et al., 2008). The presence of conspecifics can influence the movement of individuals in various ecological contexts (e.g., Delgado et al., 2014). Additionally, during preliminary observations several black rockfish started to move with 1–4 other individuals when 16 individuals were simultaneously released (Takagi, personal observation; see Fig. 4-1). For most fish species, sight and the lateral line sense are the primary sources of social information (Partridge and Pitcher, 1980; Faucher et al., 2010; Strandburg-Peshkin et al., 2013). If black rockfish recognize other conspecifics with their sensory organs, presumably their movements could be motivated or influenced at least partially by those of conspecifics. Thus, there is a possibility that adult black rockfish released in a group move together, i.e., that they use social cues from conspecifics for navigation during their homing journey.

Therefore, I developed the following two hypotheses: (1) black rockfish released with other individuals would return to their original habitat after artificial displacement while forming a group; and (2) group

homing would improve the efficiency of homing compared to that performed by a single homing fish.

## 1.3 Monitoring methods

### 1.3.1 Biotelemetry technique

Recent advancements in information and communication technology (ICT) have produced various methods for the transfer of information across the globe, from the deep sea to space. These technological advances have also helped to enhance the study of animal movement and behaviour. Acoustic biotelemetry using ultrasonic waves to send information underwater is a powerful tool that can be used to indirectly observe the behaviour and surrounding environment (e.g., depth, acceleration, conductivity, and temperature) of target animals. Technological advances in biotelemetry techniques, such as miniaturization, battery engineering, and software/hardware developments, have led to increased attention on aquatic animals, including fish, by applying these techniques (Cooke et al., 2004; Hussey et al., 2015; Hays et al., 2016). Transmitters generally have a unique ID encoded in their signals, which allows several tagged animals to be monitored. Although previous studies using biotelemetry examined the movement and behaviour of many fish, movement analyses have still largely focused on single, individual fish. There is limited information on conspecific interactions, namely group behaviours (e.g., schooling and shoaling), despite group behaviour being widespread among fish.

There are still difficulties that prevent biotelemetry systems from being used to monitor collective movement. Conventional biotelemetry systems cannot simultaneously identify multiple signals because of the relatively long duration of signals and interference between multiple monotone pulses. For example, coded Vemco transmitters each have a single frequency and a pulse duration of 10–20 ms. One signal includes 7–11 pulses, and the signal duration is 3.5–4 s (Bowles et al., 2010; for details,

see Pincock, 2008). All pulses in a signal must be detected to confirm the transmitter's ID because the coded information is contained within the intervals between pulses. As another example, Sonotronic's telemetry transmitters have several frequencies, and their signals include multiple pulses (for details, see http://www.sonotronics.com). Accordingly, several seconds are needed to broadcast a coded signal, and all pulses in the signal must be detected to confirm the ID of the signal. Owing to this constraint, many signals collide among pulses if several transmitters are broadcasting at the same time. The number of signals identified will thus be underestimated if several tagged individuals occur within the detection range of the receiver simultaneously.

## 1.3.2  Acoustic positioning

Using biotelemetry techniques, the movements and behaviours of aquatic animals, including fish, have been observed by locating the exact positions of animals using the hyperbolic positioning method, which is based on the time difference of arrival (TDOA) of the transmitted signals received by three or more stationary receivers (hydrophones). Acoustic positioning methods have also advanced to meet the challenges posed by observing the exact positions of aquatic animals to achieve an accuracy of several metres (see Cote et al., 1998; Klimley et al., 2001; Andrews et al., 2011; Espinoza et al., 2011; Gergé et al., 2012; Roy et al., 2014). These positioning methods have revealed the detailed behaviours and movements of tagged individuals using positional information, such as homing behaviour (Mitamura et al., 2009; 2012) and movement patterns (Wolfe and Lowe, 2015; Williams-Grove and Szedlmayer, 2016).

Generally, two types of biotelemetry system have been used in positioning techniques, specifically those in which receivers (hydrophones) are either wired or wireless. Both types have advantages and disadvantages in comparison to one other. On the one hand, an advantage of the wired type is that it is not necessary to synchronize the internal clocks of receivers

because all hydrophones are usually hardwired to a single receiver's main body; however, a disadvantage is that with wired types the range of the hydrophone array is limited, because it is restricted by the cable length. Due to these characteristics, a wired type of biotelemetry system has been applied to monitoring the movement and behaviour of fish within limited areas, such as around a dam or in a fish pen (e.g., the HTI [Hydroacoustic Technology Inc., Seattle, WA, USA] high resolution 3D positioning system; Ransom et al., 2008; Leclercq et al., 2018). On the other hand, the wireless type has an advantage in that it can be deployed over a wider range because each receiver is independent of all others. However, instead the internal clocks of the receivers must be manually synchronized. Although the necessity of clock synchronization is a limitation of the wireless type, the majority of biotelemetry systems used in the field have been of the wireless type because studies have needed to be conducted over wider ranges, in environments ranging in size from ponds to the deep sea (Hussey et al., 2015). The Vemco Positioning System (VPS) is one of the wireless types that has been particularly widely-used in biotelemetry studies (e.g., Andrews et al., 2011; Espinoza et al., 2011). However, both the wired and wireless types of receivers only have positioning accuracies of up to several tens of centimetres, which does not reach the level of accuracy required to observe the collective movement of small fish of a few tens of centimetres in length, such as the target species.

## 1.3.3 New biotelemetry system

Recently, several biotelemetry systems that can identify multiple signals at a time have been developed. For example, HTI has developed a biotelemetry system known as the Predation Detection Acoustic Tag (PDAT), which uses a phase modulation pulse. Individual PDATs are isolated by 'pulse-rate encoding', which uses only one phase modulation pulse, a programmable short pulse (0.5–5 ms), and a unique pulse repetition rate (for details, see Ehrenberg and Steig, 2003). The short-phase modulation pulse allows

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receivers to detect multiple signals simultaneously. As another example, CDMA (code division multiple access) MAP technology (Lotek Wireless Inc., Newmarket, Ontario, Canada) can detect transmissions from 22 tagged fish at 15 s intervals, and localise the fish with sub-metre accuracy (Cooke et al., 2005).

However, I focused on and used another biotelemetry system in my research. Miyamoto et al. (2010, 2011) developed a new biotelemetry system (Fusion Inc., Tokyo, Japan) for use in the ocean from the temperate zone to the tropics, especially where there are high ambient noise levels. This new biotelemetry system attained the four parameters that are desired in an optimum biotelemetry system, specifically: small size, long battery life, long-distance transmission, and high recognition ability. The biotelemetry system that I used in my thesis research was the successor to the model developed by Miyamoto et al. (2010, 2011), which was named the Gold code transmitter and receiver system and was manufactured by Aquasound Inc., Kobe, Japan (Fig. 1-2).

**Transmitter**

Three types of Gold code transmitters were used in my research; AQPX-1030P, AQPX-1040PT, and AQTD-600P (AquaSound Inc., Kobe, Japan; Fig 1-2). The Gold code transmitters emit a remarkably short (2.048 ms) pulse, which is implemented as single-frequency phase-modulated pulses using M-sequence codes that generate a high signal-to-noise ratio (SNR). These pulses are each encoded by one of 32 Gold codes, which is one of the pseudo-noise (PN) codes in a pair of M-sequences, with low cross-correlation among the codes (Gold 1967). Adequately short (2.048 ms) pulses enable the incidence of collisions among multiple pulses at a time to be decreased. In practice, three or four collisions occurred over 1 min when seven transmitters with a transmitting interval of ~1 s were placed in a 30-cm-diameter bucket (Miyamoto et al., 2011). The low cross-correlation of Gold codes decreases their misidentification rates. Therefore, these two strong characteristics (lower collision rate and lower misidentification rate)

would facilitate simultaneous identification of multiple signals, i.e., multiple fish tagged with the Gold code transmitters.
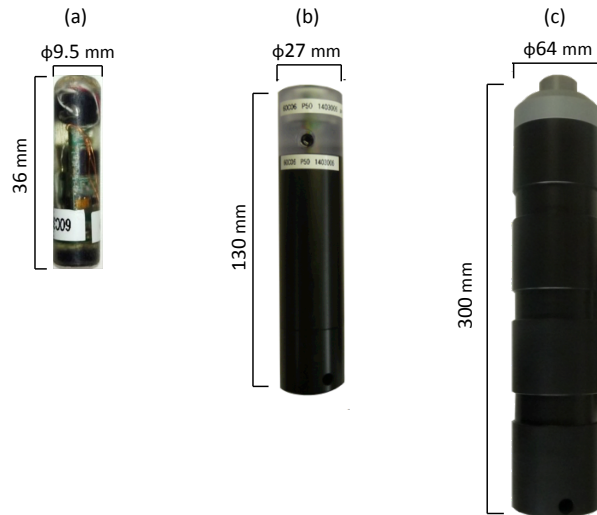


**Fig. 1-2.** Size of transmitters and receivers of the biotelemetry system used in this study. (a) A transmitter of either the AQPX-1040PT or AQPX-1030P type (Aquasound Inc., Kobe, Japan). (b) A transmitter of the AQTD-600P type (ditto). (c) A receiver of the AQRM-1000 type (ditto).

The Gold code transmitters can also transmit ambient environmental information. The AQPX-1030P transmitter is equipped with a pressure (depth) sensor, while the AQPX-1040PT and AQTD-600P transmitters are equipped with both pressure (depth) and temperature sensors. A signal emitted by the transmitters consists of two (with depth sensor only) or three (with depth and temperature sensors) consecutive 2.048 ms pulses. Depth and temperature information are encoded in intervals between the 1st and 2nd, and the 2nd and 3rd pulses, respectively (Fig. 1-3). The accuracies of these systems were 0.5 m (depth sensor) and 0.2 °C (temperature sensor).
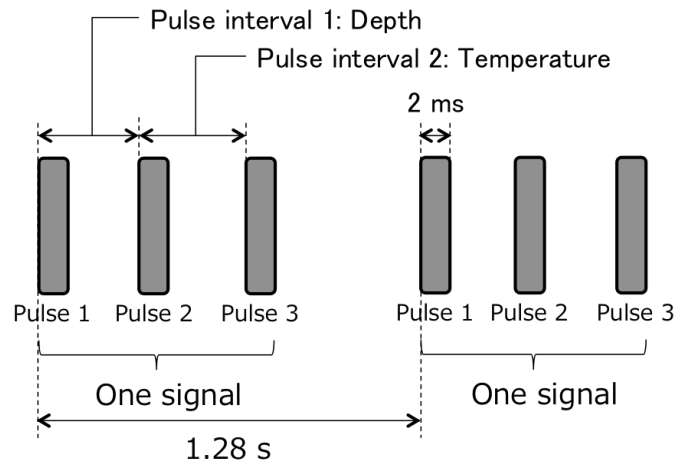
**Fig. 1-3.** Transmission of depth and temperature information from the Gold code transmitter. Depth information is included in the 1st and 2nd pulses, and temperature information is included in the 2nd and 3rd pluses. The signal interval is assumed to be 1.28 s. In transmitters that have only a depth sensor, a signal consists of two pulses, i.e., the 1st and 2nd pulses.

Other features of the Gold code transmitters are as follows. The AQPX-1030P and AQPX-1040PT transmitters measured 9.5 mm in diameter and 36 mm in length, and weighed 1.6 g in water and 5.5 g in air. The source level of these two transmitter types was 155 dB re 1 µPa at 1 m, and their frequency was 62.5 kHz. The battery life of AQPX-1030P and AQPX-1040PT transmitters was 1 year when emitting signals at 180 s and 240 s transmitting intervals, respectively. The AQTD-600P transmitters were 27 mm in diameter and 130 mm in length, and weighed 93 g in water and 17 g in air. The source level of the AQTD-600P transmitter type was 160 dB re 1 µPa at 1 m, and its frequency was 62.5 kHz. Its battery life was 1 year when emitting signals at 5 s transmitting intervals using a single CR2 lithium cell.

**Receiver**

In my research, AQRM-1000 acoustic monitoring receivers (AquaSound Inc., Tokyo, Japan; Fig. 1-2) were used. They measured 64 mm in diameter and

300 mm in length, and weighed 0.5 kg in water and 1.2 kg in air when containing three D-cell batteries. The receivers had a battery life of approximately two months, and stored detection data in CSV format on an SD card, from which the data were then retrieved and downloaded for analysis.

The receivers were theoretically capable of simultaneously identifying multiple pulses from different transmitters using cross-correlation values. Additionally, as the receiver has a sampling frequency of 1 MHz, the theoretical positional resolution baseline was only 1.5 mm (calculated by dividing the underwater sound velocity [$1,500 \text{ m} \cdot \text{s}^{-1}$] by 1,000,000 Hz) based on the time-difference-of-arrival (TDOA). This biotelemetry system should thus be capable of simultaneously and precisely observing the movements of multiple fish, a feat that conventional telemetry positioning systems with sub-metre accuracy have not previously achieved.

## 1.4 Objectives of this research

The ultimate objectives of this research were to test the two hypotheses mentioned in the previous section by acoustic positioning using a new biometry technique. To accomplish this objective, the three main steps in this study were set as follows:

1. Empirically evaluate the ability of the biotelemetry system used in this study to simultaneously identify multiple targets in a natural environment.

2. Using the biotelemetry technique, develop a three-dimensional acoustic positioning method that can simultaneously localize multiple fish at spatial and temporal scales as fine as those needed for observing the movement behaviour of fish schools.

3. Observe homing behaviour of multiple black rockfish simultaneously using the acoustic positioning method developed herein, and test the two main study hypotheses with the positional dataset acquired.

## 1.5 Structure of the thesis

This thesis is organized as follows:

- Chapter 2:
  I describe a demonstration of the ability of the biotelemetry system used in this study to simultaneously identify targets while avoiding multi-path interference effects in a natural environment.

- Chapter 3:
  I describe the development and application of a fine-scale three-dimensional positioning method using the biotelemetry system in a natural environment, which I apply to the observation of intermittent locomotion by a coastal fish species, Siebold's wrasse (*Pseudolabrus sieboldi*).

- Chapter 4:
  I describe the application of the positioning method to observing and testing hypotheses about the collective movement behaviour of the target coastal fish species, the black rockfish *S. cheni*, while homing back to original habitats following displacement.

- Chapter 5:
  Based on the results of Chapters 2–4, I discuss the potential contribution of the positioning method developed herein to monitoring group behaviour in fish in future research, and conclude this thesis.

# Chapter 2

# Ability for simultaneous identification of the biotelemetry system in natural environment

## 2.1 Introduction

Acoustic biotelemetry using ultrasonic transmitters and receivers is a powerful tool to observe indirectly the behavior and surrounding environment (e.g., swimming depth and ambient water temperature) of target animals in water, so a large number of studies have been performed on aquatic animals using this advanced technology (Cooke et al., 2004; Hussey et al., 2015). Ultrasonic biotelemetry methods have revealed various fish behaviors and movements. For example, our previous studies reported that black rockfish *Sebastes cheni* exhibit strong homing behavior. Rockfish return to their original source after displacement, indicating that they may predominantly use olfaction during homing (Mitamura et al., 2002, 2005). Furthermore, the behaviors of aquatic animals including fish have been observed by locating the exact positions of animals tagged with transmitters using the hyperbolic navigation method based on the time difference of arrival of the transmitted signals with three or more stationary receivers. The Vemco Positioning System (VPS) has been widely used to locate tagged

animals with positioning accuracy of a few meters (Andrews et al., 2011; Espinoza et al., 2011). Transmitters generally have a unique ID encoded in their signals. The coded transmitter allows several tagged animals to be monitored. Although those biotelemetry studies examined the behavior of many fish, the analyses were focused on the behavior of individual fish, and little attention was paid to inter-individual interactions, namely, group (e.g. schooling and shoaling) behavior, in biotelemetry studies, despite group behavior being widespread among fish.

Several studies have attempted to develop methods to observe fish group behavior. Guttridge et al. (2010) tagged juvenile lemon sharks (*Negaprion brevirostris*) with a transmitter and a receiver (Sonotronics, Tucson, AZ, USA) and demonstrated that one shark associated with nine other sharks on 128 occasions over 17 days. Holland et al. (2009) developed "business card" tags (BCT; Vemco, Bedford, NS, Canada), consisting of a transmitter and a receiver, and tagged Galapagos sharks (*Carcharhinus galapagensis*) with the BCTs. The BCTs captured presence-absence characteristics of other tagged sharks within the detection range.

However, these telemetry systems cannot simultaneously identify multiple signals because of the relatively long signal duration and plural monotone pulses. For example, the coded Vemco transmitters used in BCTs (Holland et al., 2009) have a single frequency and a pulse duration of 10–20 ms. One signal includes 7–11 pulses, and the signal duration is 3.5–4 s (Bowles et al., 2010; for details, see Pincock, 2008). All pulses in a signal must be detected to identify the ID because the coded information is contained within the intervals between pulses. Sonotronics telemetry transmitters have several frequencies, and their signals include multiple pulses (for details, see http://www.sonotronics.com). Accordingly, several seconds are needed to broadcast a coded signal, and all pulses in the signal must be detected to identify the ID. Owing to this constraint, many signals collide among pulses if several transmitters are broadcasting signals at the same time. The number of signals identified will be underestimated if several tagged individuals occur within the detection area of the receiver

simultaneously. Short duration interactions may not be recorded by BCT (Holland *et al.,* 2009)-like inter-animal tags or the telemetry system used by Guttridge *et al.* (2010). A biotelemetry system with monotone pulses cannot detect signals or identify IDs simultaneously; namely, it is difficult for this widely used system to detect group behavior on a precise time scale.

Multiple signals from several transmitters must be detected simultaneously and discriminated by receivers to obtain a better understanding of group behavior using biotelemetry. HTI (Hydroacoustic Technology Inc., Seattle, WA, USA) has developed the Predation Detection Acoustic Tag (PDAT), which uses a phase modulation pulse. Individual PDATs are isolated by "pulse-rate encoding", which uses only one phase modulation pulse, a programmable short pulse (0.5–5 ms), and a unique pulse repetition rate (for details, see Ehrenberg and Steig, 2003). The short phase modulation pulse allows receivers to detect signals simultaneously. Predation has been detected by locating dead juvenile Chinook salmon (*Oncorhynchus tshawytscha*) with a PDAT and a predator with another tag after almost 2 days (Schultz et al., 2015). Miyamoto et al. (2010, 2011) developed a new telemetry system (Fusion Inc., Tokyo, Japan) in which the transmitters emit short (2 ms), phase-modulated pulses using M-sequence signals, and the receivers correlatively process the data. The pulses are encoded by one of 32 Gold codes, which is one of the pseudo-noise codes in a pair of M-sequences, with low cross-correlation among the codes (Gold 1967). The new system theoretically detects signals and identifies the IDs simultaneously because each pulse contains a unique ID, and the short pulses rarely collide. Miyamoto et al. (2011) confirmed that three or four collisions occurred over 1 min when seven transmitters with an approximate 1-s transmitting interval were placed in a 30-cm-diameter bucket. However, little is known about whether multiple signals from plural phase modulation transmitters can be identified simultaneously in a natural environment. It is essential to investigate the ability of the system to identify signals simultaneously under natural conditions to observe group behavior of fish by using the BCT-like inter-animal tags and a

VPS-like positioning system.

Another problem would occur in the new biotelemetry system. The Gold code transmitters emit three consecutive 2 ms pulses to encode depth and temperature information in intervals between the 1st and the 2nd, and the 2nd and the 3rd pulses, respectively. Due to its short pulse duration of 2 ms and pulse intervals ranging between a few tens of ms, there is a multipath effect that causes measurement errors in coded information. If one of the two pulses were a reflected pulse, the interval between the two pulses would become shorter or longer; that is, correct encoded information would collapse and phantom values might be recorded. Hasegawa *et al.* developed a real-time depth monitoring system for trolling gear using a type of the Gold code transmitters, and proposed two ways to solve the problems of multipath effects (Hasegawa et al., 2016). One method was adding directivity to the hydrophone to detect only direct pulses, while the other method was deploying the hydrophone at the bottom of the boat to block the reflected pulses at the sea surface. The trolling gear with the Gold code transmitter was submerged under the boat and the hydrophone was deployed around the boat, so the pulses from the Gold code transmitter were almost always propagated in an upward direction. The cause of the phantom value in that study was the reflected pulse at the sea surface. Therefore, using a directional hydrophone and deploying the hydrophone under the boat should solve the problems.

However, the two methods in fact may not solve the problems in biotelemetry. The majority of research using acoustic biotelemetry has been performed in shallow waters such as coastal, estuarine, and freshwater areas (Hussey et al., 2015), where signals from transmitters to receivers mainly propagate in horizontal directions due to limitation of depth range. Animals tagged with a transmitter are free ranging, so signal arrival direction is always unknown. Therefore the two methods mentioned above cannot be applied because those methods work only in when the direct or reflected signal arrival direction is known. Additionally, in shallow waters, not only sea surface but also sea bottom can be major boundaries to reflect

ultrasonic pulses.

One possible way to overcome this problem is to discriminate phantom values through a mechanical process. If both a receiver and a transmitter remain stationary under a condition of flat sea surface, direct and reflection waves always travel through the same passage, determined geometrically. As a result, phantom value derived from false alarms would concentrate around a specific value, because time difference of arrival between direct and reflection wave is always the same. However, the sea surface is rarely so calm as to be flat, and tagged animals are free-ranging in biotelemetry, so phantom values should not be uniquely decided. These factors make it difficult to cope with the multipath effect through a mechanical process. Thus, al least, it is necessary to evaluate measurement errors derived from the multipath effect in the shallow sea area, where majority of research on biotelemetry was conducted.

In this study, I (1) examined whether multiple signals from plural phase modulation transmitters could be identified simultaneously, and (2) evaluated the multipath effects on depth measurement using a new biotelemetry system under natural conditions.


## 2.2 Materials and methods

### 2.2.1 Biotelemetry system

The transmitters used in this study were Gold code transmitters equipped with pressure (depth) and temperature sensors (AQPX-1040PT; AquaSound Inc., Kobe, Japan; 9.5 mm in diameter and 36 mm in length, 1.6 g in water) broadcasting signals consisting of three consecutive 2-ms pulses. The source level of the transmitters was 155 dB re 1 µPa at 1 m, and the frequency of the transmitter was 62.5 kHz. The acoustic monitoring receivers (AQRM-1000; AquaSound Inc., Tokyo, Japan; 64 mm in diameter and 300 mm in length) were capable of simultaneously identifying multiple pulses from the plural transmitters using cross-correlation values. The receivers

stored detection data in CSV format on an SD card. Data was then retrieved and downloaded for analysis. Receivers had a battery life of approximately two months with three D-size cells.

## 2.2.2 Field experiment

A field experiment was conducted in a shallow sea area (bottom depth, approx. 10 m) near Kaminokae fishing port, Kochi, Japan, on August 19, 2015, because we assumed that shallow water would be an important area to observe group fish behavior. Water temperature in the experimental area was 27.6°C. Eight transmitters with unique IDs were deployed at a fixed position, with a signal interval of 1.28 s. Three (IDs: C1, C2, and C3) of the eight transmitters were 3.0 m deep, three were (ID: C4, C5, and C6) placed at 4.0 m, and the remaining two (ID: C7 and C8) were placed at 5.0 m (Fig. 2-1). Two receivers were deployed at a depth of 2.0 m and were 18.5 m (R1) and 38.5 m (R2) horizontally away from the transmitters, considering the mean horizontal size of the fish schools observed around set-nets using scanning sonar, namely, 37 m in length and 15 m in width (Inoue, 1987). The reason the receivers were not deployed near the sea surface was to avoid unfavorable environmental conditions for the hydrophones, such as the signal-to-noise ratio (SNR) deterioration due to the noise induced collapse of waves by wind and exposure to the air due to vertical vibration of the sea surface. The horizontal distance between the receivers and the transmitters was sufficient for inter-individual tagging studies. The actual detection range varied across the study area, period, and season. Although the distances used might be conservative for VPS-like studies, the evaluation of simultaneous identification at relatively short distances was adequate for the purpose of this study. The bottom depths of R1, R2, and the transmitters were 9.1, 8.8, and 9.3 m, respectively. Recording duration was 15 min. The detection rates and duration of the transmitters were calculated as a percentage of the number of signals recorded by the receiver to the number of signals emitted by the transmitter, which was estimated

by dividing duration (15 min = 900 s) by the signal-transmitting interval (1.28 s). Mean recorded signal intervals were calculated by dividing the duration by the number of signals recorded.

Measurement accuracies of the depth of each transmitter by R1 and R2 were defined as mean of absolute values of difference between the recorded depths and the actual installation depths. Only the depth information, except for temperature information, was used for further analyses. The depth data were categorized into the following 4 groups:

(A) *Correct values:*

The correct values range within plus or minus 0.15 m to the average depths of each transmitter in accordance with the resolution of depth censor in the transmitters.

(B) *Phantom values caused by reflection at the sea surface, and*

(C) *Phantom values caused by reflection at the sea bottom:*

By using the installation depths of the transmitters and the receivers, the distances between them, and a sound velocity (1500 m/s), time difference of arrival between direct and reflected pulse at the sea surface and bottom were estimated, assuming that a pulse was reflected only once at either of the boundaries. Two types of measurement depth affected by the reflection were then calculated – the measurement depth between the direct 1st pulse and the reflected 2nd pulse, and between the reflected 1st pulse and the direct 2nd pulse. The phantom values caused by reflection at the sea surface and bottom were within plus or minus 0.30 m of the measurement depth affected by the reflection of each transmitter.

(D) *Other values:*

The remaining values that did not match with the estimated values of cases (A), (B) and (C) were categorized into 'other values'
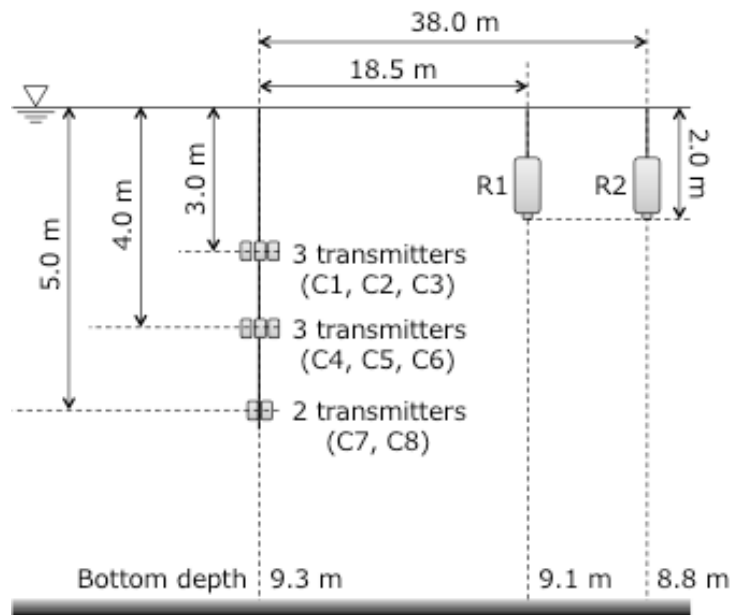
**Fig. 2-1.** Schematic drawing of the vertical profile arrangement of the eight transmitters (C1–8) and the two receivers (R1 and R2) in the experiment.

## 2.3 Results

Median R1 and R2 detection rates were 77.0% (range: 57.6–81.4; N = 8) and 73.0% (range: 54.7–90.3; N = 8), respectively (Fig. 2-2). The mean recorded signal intervals were 1.67 s for R1 and 1.76 s for R2. The mean accuracy of the depth measurement was $0.38 \pm 0.37$ m for R1 and $0.46 \pm 0.50$ m for R2. Mean proportions of categorized depth data to the total number of the depth data recorded in R1 were (A) $88.4 \pm 6.5\%$, (B) $8.1 \pm 4.5\%$, (C) $0.6 \pm 0.9\%$, and (D) $2.9 \pm 2.5\%$. Those values in R2 were (A) $86.3 \pm 4.8\%$, (B) $3.2 \pm 2.5\%$, (C) $5.3 \pm 3.2\%$, and (D) $5.2 \pm 2.0\%$ (Fig. 2-3).
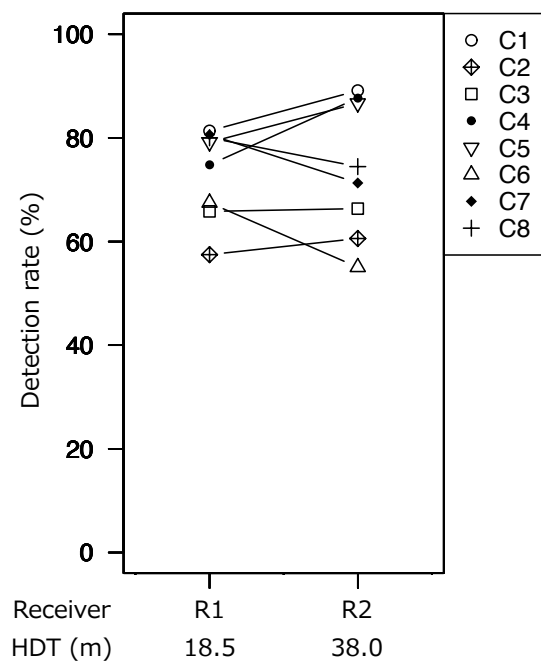


**Fig. 2-2.** Detection rates (%: number of signals recorded by each receiver/number of signals emitted by each transmitter × 100) of signals transmitted from the eight transmitters (C1–8) to the two receivers (R1 and R2). The horizontal distances from the transmitters (HDT) to R1 and R2 were 18.5 m and 38.0 m, respectively.
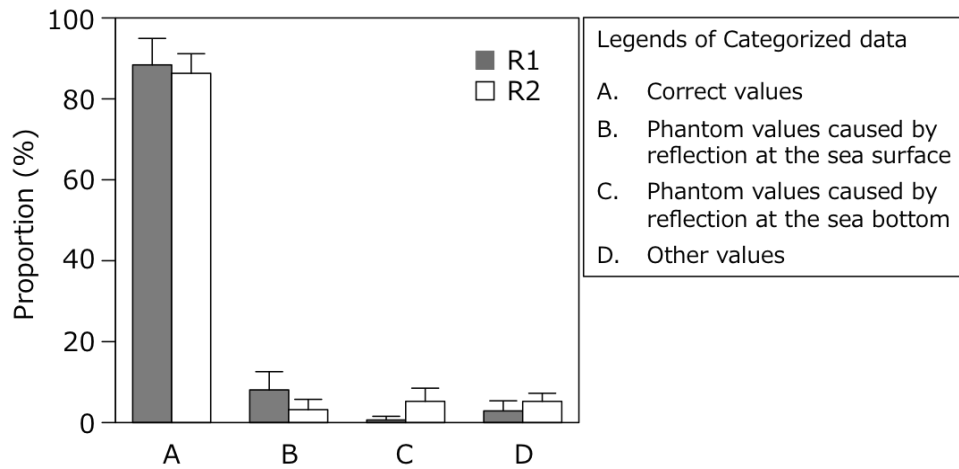
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**Fig. 2-3.** Proportions of four types of categorized depth measurements (Mean + SD, N = 7 transmitters) recorded in receiver1 (R1) and receiver2 (R2). Horizontal distances between the transmitters and R1, and R2 were 18.5 m, and 38.0 m, respectively.

## 2.4 Discussion

The results show that multiple signals from plural transmitters were detected simultaneously and discriminated by receivers deployed at distances of the mean size of a fish school. The mean recorded intervals in this study were so short that data were collected with adequately high time resolution for observing fish group behavior. It is difficult to obtain such high frequency resolution data with a widely used telemetry system and monotone transmitters. A detection rate < 100% might be caused by environmental noise, opportunistic collisions, or false detection. The detection rates of certain signals from the transmitters tended to be lower in both R1 and R2 (Fig. 2-2). The variability in the detection rates among the eight transmitters may have also resulted from those factors. However, the signals from the telemetry system used in this study rarely collide (Miyamoto et al., 2011), and environmental noise would equally affect all transmitter signals. It is unlikely that the false detection rate of a

particular transmitter was much higher or lower than that of another due to a systematic receiver malfunction. The reasons for the different detection rates should be clarified in a future study.

Unlike Hasegawa et al. (2016), the phantom values originated from the pulses reflected at not only the sea surface but also the sea bottom (Fig. 2-3). This may have resulted from propagation characteristics in shallow waters with multipath effects. Other values might include a plurality of reflections at both surface and bottom. Nonetheless, accuracy of depth measurements was $0.38 \pm 0.37$ m for R1 and $0.46 \pm 0.50$ m for R2, which was considerably high. Additionally, proportion of correct values ($88.4 \pm 6.5\%$ in R1, and $86.3 \pm 4.8\%$ in R2) occupied a majority of the acquired data, and apparently higher than phantom values from the surface ($8.1 \pm 4.5\%$ in R1, and $3.2 \pm 2.5\%$ in R2) and from the bottom ($0.6 \pm 0.9\%$ in R1, and $5.3 \pm 3.2\%$ in R2), and also other values ($2.9 \pm 2.5\%$ in R1, and $5.2 \pm 2.0\%$ in R2; Fig. 2-2). Those results suggest that the biotelemetry system would provide accurate depth information.

The new telemetry system with phase modulation transmitters and BCT (Holland *et al.*, 2009)-like inter-individual tags allowed us to observe group fish behavior at a high time frequency resolution. The BCTs (Holland *et al.*, 2009) and the telemetry system used by Guttridge et al. (2010) were unable to detect short interactions between animals and could not detect the ID under a fish schooling condition because of a limitation of monotone transmitters. BCTs (Holland et al., 2009) were not designed to detect interactions when animals are in brief proximity to each other. A single transmitter used by Guttridge et al. (2010) theoretically took 3–4 s to be identified, and many other transmitters would take longer to be identified due to colliding signals. However, the mean recorded signal intervals were < 2 s in this study, so the inter-individual tags with phase modulation transmitters allowed us to obtain data showing when they met each other, how long they were together, and when they parted. Schooling, aggregating, and dispersing behaviors were observed when several fish of the same species were tagged, although the exact locations where the behavior

occurred could not be pinpointed.

On the other hand, the VPS-like positioning system allowed monitoring of the positions of many individuals because of the simultaneous signal identification capability. For example, Masuda et al. (2003) showed the development of schooling behavior in larval and juvenile Spanish mackerel *Scomberomorus niphonius,* and Marras et al. (2014) reported that any individual fish in a school gains an energy benefit compared with swimming alone, regardless of their spatial position. Those studies were conducted in laboratory tanks. Group behavior in a natural environment can be further understood using the new telemetry system with phase modulation transmitters by observing inter-individual interactions, such as distance, relative speed, and angles, among individual fish. A large number of artificially propagated seedlings are released in one location for stock enhancement studies. For example, Wada et al. (2014) released 27,900 and 53,217 spotted halibut *Verasper variegatus* seedlings into a shallow brackish lagoon (Matsukawa-ura) in 2003 and 2004, respectively. However, it was difficult to monitor their movement just after release using monotone transmitters. The new telemetry system would determine the movements of these artificially propagated seedlings after release; thus, actual survival rates and preferable release sites could be determined. If both predator and prey were tagged with transmitters, predator-prey interactions could be observed on a more precise spatiotemporal scale. Furthermore, it would be possible to observe courtship and territorial behaviors.

This study shows the possibility of better understanding fish group behavior and movement using a new biotelemetry system with phase modulation transmitters. This system will be increasingly applied in studies on fish schooling behavior, movements of many fish in a limited area, and inter- and intra-specific interactions in the future.

## 2.5 Conclusions

In this chapter, I described ability of a biotelemetry system used in this study, which can simultaneously identify multiple signals in natural environment. This biotelemetry system will have an ability to observe multiple aquatic organisms at precise time scale. In the next chapter, development of fine–scale 3-dimensional positioning method using this biotelemetry system is introduced.

# Chapter 3

# Development and application of fine-scale spatiotemporal positioning method in natural environment

## 3.1 Introduction

Biotelemetry techniques have been widely used in the past two decades to monitor the behaviour and movements of many aquatic organisms (Cooke et al., 2004; Hussey et al., 2015; Hays et al., 2016). Acoustic positioning methods using ultrasonic transmitters and moored receivers have also advanced to meet the challenges posed by observing the exact positions of aquatic organisms, including fish, with accuracy of approximately 3−10 m based on a hyperbolic positioning technique (see Cote et al., 1998; Klimley et al., 2001; Andrews et al., 2011; Espinoza et al., 2011; Gergé et al., 2012; Roy et al., 2014). These positioning methods have revealed the details of behaviour and movements of tagged individuals using positional information [e.g. homing behaviour (Mitamura et al., 2009; 2012) and movement patterns (Wolfe and Lowe, 2015; Williams-Grove and Szedlmayer, 2016)]. Behavioural intermittence (e.g. stops, or drastic changes in speed) is also a popular topic in movement ecology. Behavioural intermittence is significant to animal locomotion during activities (e.g.

feeding, directed movements toward a nest, or habitat assessments) because sharp reorientations often follow the intermittence (Bartumeus, 2007; Bartumeus and Levin, 2008). To observe the intermittence of aquatic organisms in a limited area, their positions at fine-scale spatiotemporal resolutions should be obtained. However, there are many challenges to tracking aquatic organisms at finer-scale temporal (5−10 s) and spatial (<1 m) resolutions. This is because conventional telemetry systems require a wider signal-transmitting interval (>30 s) to reduce collisions between received signals (Løkkeborg et al., 2002), and because the positioning accuracy of such systems is merely 3−10 m (see Cote et al., 1998; Klimley et al., 2001; Andrews et al., 2011; Espinoza et al., 2011; Gergé et al., 2012; Roy et al., 2014).

Recently, telemetry systems have been developed to enable the simultaneous tracking of multiple fish at higher temporal resolutions. For example, CDMA MAP technology can localize ~75% of transmissions from 22 tagged fish at 15 s intervals with sub-metre accuracy, and was used to develop a spatial histogram of tagged fish in a small lake (9.2 ha) based on their observed positions (Cooke et al., 2005). A new biotelemetry system consisting of phase modulation-coded transmitters ('Gold code transmitter') and receivers, developed by Miyamoto et al. (Miyamoto et al., 2010; 2011), can simultaneously identify ~75% of multiple coded signals at a transmitting interval of 1.28 s, which was demonstrated in the previous chapter. As the receiver has a sampling frequency of 1 MHz, the theoretical baseline positional resolution will be only 1.5 mm (dividing the underwater sound velocity 1,500 m・s$^{-1}$ by 1,000,000 Hz) based on the time-difference-of-arrival (TDOA). This biotelemetry system should thus be capable of simultaneously observing the precise movements of multiple fish, a feat that conventional telemetry positioning systems with sub-metre accuracy have not achieved. However, the positioning performance of this new biotelemetry system has never been examined.

In this chapter, a simultaneous positioning system with high spatiotemporal resolution using Gold code transmitters and receivers was

proposed. In order to evaluate the positioning performance of the proposed system, multiple stationary transmitters were firstly localised. Then, movement of free-swimming coastal fish (Siebold's wrasse *Pseudolabrus sieboldi*) was simultaneously observed for application of the proposed system. Subsequently, the stop-and-go behavioural intermittence of the fish homing to their original location from an unsuitable location was observed.

## 3.2 Materials and methods

The Director-General of the Hiroshima Prefectural Agriculture, Forestry, and Fisheries Station issued permission for fish collection for study in this chapter around Ikuno Island. All procedures including the sampling protocol and the tagging surgery in this study were approved by the Animal Research Committee of Kyoto University (permit number: Inf-K15003).

### 3.2.1 Biotelemetry system

Two types of Gold code transmitters and acoustic monitoring receivers (AQRM-1000, AquaSound Inc., Kobe, Japan; 64 mm in diameter and 300 mm in length), successors to the models developed by Miyamoto et al. (Miyamoto et al., 2010; 2011), were used in this study. Gold code transmitters transmit signals consisting of three consecutive 2 ms short pulses coded by one of 32 Gold codes at a frequency of 62.5 kHz. Gold codes are single pseudo noise (PN) codes in pairs of M-sequences, which can decrease the false identification rate (Gold, 1967). Pressure and temperature sensors were incorporated into the Gold code transmitters, allowing swimming depth and ambient temperature information to be encoded in the intervals between the 1st and 2nd, then 2nd and 3rd pulses, respectively. The system accuracies were 0.5 m (pressure sensor), and 0.2 °C (temperature sensor). Temperature data observed was not used for analysis in this study because it was unnecessary for evaluating the positioning performance. The acoustic monitoring receiver simultaneously

identified multiple pulses from multiple transmitters at once using cross-correlation values (Takagi et al., 2016). The receivers stored detection data in CSV format on an SD card. Data was then retrieved and downloaded for analysis. Receivers had a battery life of approximately two months with three D-size cells.

## 3.2.2 Study site

Field experiments were conducted in a shallow sea area near the east coast of Ikuno Island (34°29'N, 132°92'E) in the Seto Inland Sea, Japan, in October 2015 (Fig. 3-1a–b). The depth of the seafloor in the area was separated into shallower (<4 m) and deeper (>8 m) levels by steep gradients (Fig. 3-1c). The sea bottom of the area was covered with sand and mud. There were two rocky areas in the deeper area along the boundary between the two levels (Fig. 3-1c). The tidal difference during the experiments was approximately 3.5 m.

An array of seven receivers with overlapping detection ranges was deployed on the seafloor in the study area (Fig. 3-1c), covering an area of 13590.2 m². Actual receiver locations were determined using a handheld GPS unit (Garmin eTrex 30J). Seven Gold code transmitters (AQTD-600P, AquaSound Inc., Kobe, Japan; 27 mm diameter and 130 mm length) were placed, one with each receiver. The transmitters emitted signals at ~60 s intervals with a root mean square (RMS) sound pressure level (SPL) of 160 dB re 1 µPa at 1 m. The transmitter's battery life was approximately one month using a single CR2 lithium cell. The sound speed was calculated using mutual signal detections between all receiver pairs. Receiver internal clocks were synchronised using calculated sound speed, determined receiver locations, and signal TDOAs. A conservative range (~75 m) between the receivers was used to increase mutual signal detections between receivers, although a preliminary experiment showed their detection range to be >100 m.
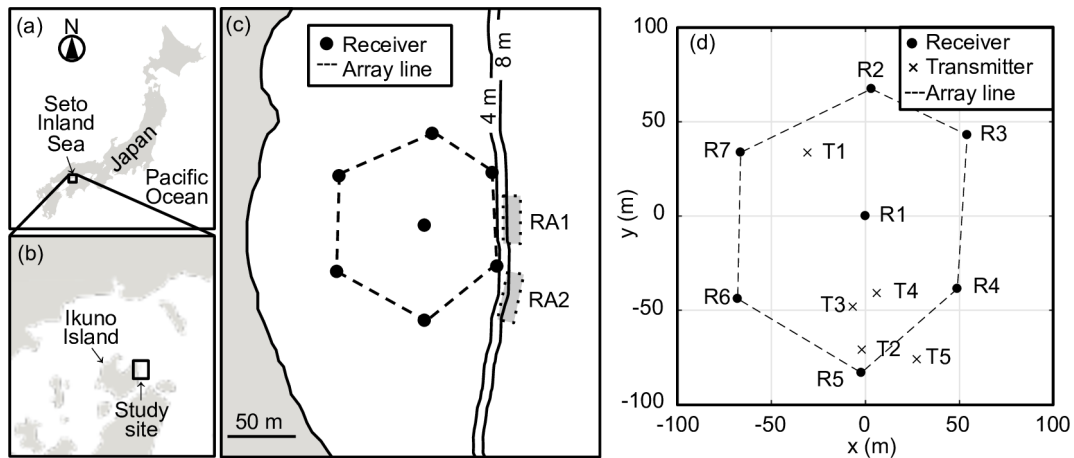
**Fig. 3-1.** Acoustic array of seven receivers and transmitters for the experiment deployed off Ikuno Island, central Seto Inland Sea, southwestern Japan. (a)–(c) The array line represents the boundary between the outside and inside of the array. Vertical lines denoted by 4 m and 8 m represent the depth contour. There were two rocky areas (RA1 and RA2) on the east side of the array, where the Siebold's wrasses used as specimens in this study were caught. (d) Seven receivers (R) and five transmitters (T) fixed on the seafloor. Horizontal and vertical axes denote the relative coordinate axes, adjusted to the central receiver (R1) of the array.

## 3.2.3 Positioning method

The horizontal and vertical components of 3D positions were obtained separately. Horizontal positions were calculated using the proposed acoustic positioning method in this study, whereas vertical positions (swimming depth) were provided by a pressure sensor installed in the transmitters. 3D positioning was not feasible because the study site had little vertical range (~2–8 m) compared to its horizontal range (~75 m).

When the three receivers detected a signal, the horizontal position of its sound source was estimated as an intersection of the three hyperbolae formed by signal TDOAs, the calculated sound speed, and the distance between the receivers for a final resolution of 1 mm (Fig. 3-2a). Six subsets of close equilateral triangles were used to estimate positions: R1, R2, and R3; R1, R3, and R4; R1, R4, and R5; R1, R5, and R6; R1, R6, and R7; or R1, R7, and R2 (Fig. 3-1d). When a signal was detected by the receivers that

included two or more subsets of the close equilateral triangles, the position was estimated using every subset (Fig. 3-2b). A single position was then selected using the shortest distance between the centroid of the estimated positions and the centroid of the close equilateral triangles used for positioning (Fig. 3-2c–d), because shorter distances imply improved horizontal delusion of precision (HDOP) values. The HDOP represents the horizontal portion of the delusion of precision (DOP), which is universally used as a positioning precision index for GPS (Langley, 1999). All positioning calculations were performed using relative coordinates modified from universal transverse Mercator (UTM) coordinates, the origin of which was adjusted to R1 (Fig. 3-1d). Positioning and spatial analyses were performed in Matlab R2017a (The Math works, Natick, Ma, USA).
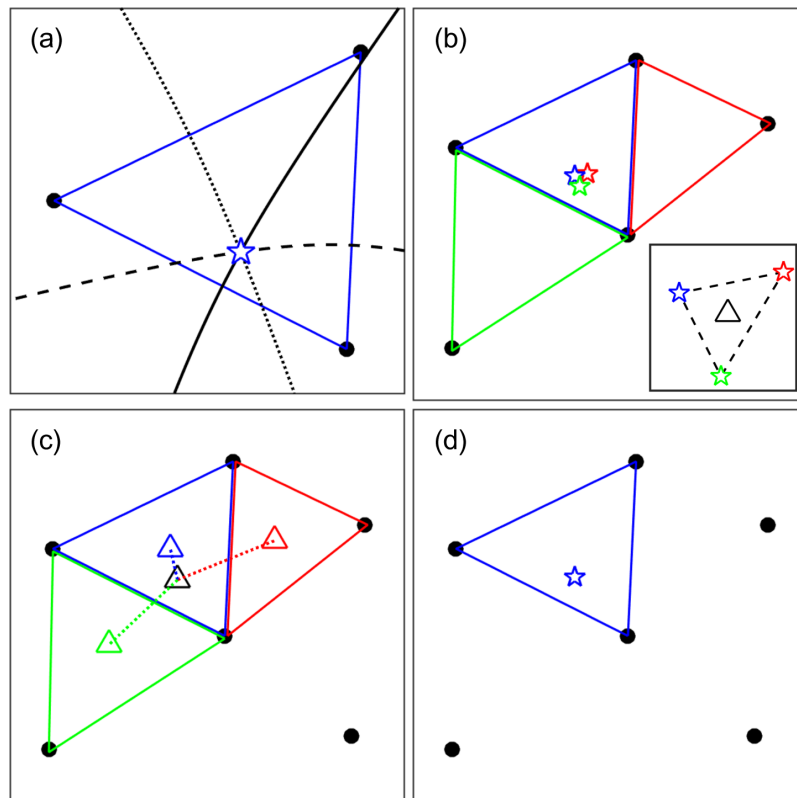
**Fig 3-2.** Example procedure of the proposed positioning method in this study. (a) A sound source position of a signal was horizontally estimated as an intersection (blue star) of three hyperbolae (bold, dashed, and dotted lines) by any subsets of close equilateral triangles. (b) When the signal was detected by five receivers forming three subsets (red, green, and blue triangles), three tentative estimates (red, green, and blue stars) were obtained by each subset. (c) Then, the distance between the centroid of three tentative estimates (small black triangle) and the three centroids of each subset (small red, green, and blue triangles) was calculated. (d) Finally, the estimated position by the blue triangle subset was selected for the final estimate using a criterion of the shortest distance.

## 3.2.4 Stationary test

The positioning performance of the proposed method was tested using stationary Gold code transmitters (AQPX-1040PT, AquaSound Inc., Kobe, Japan; 9.5 mm diameter, 36 mm length, 1.6 g weight in water) with a signal-transmitting interval of ~5.0 s and RMS SPL of 155 dB re 1 µPa at 1

m. Their battery life was approximately one week. Five Gold code transmitters were placed on the seafloor at five fixed points for 20 min. The seafloor depths of the fixed Gold code transmitter positions ranged from 2.5–2.8 m (Table 3-1). Two of them (T1 and T4) were placed near a centroid of triangulation, one (T2) near a vertex, one (T3) on a baseline, and the remaining one (T5) outside triangulation (Fig. 3-1d).

The positional precision and probability of location were calculated, allowing both the spatial and temporal resolution of the proposed positioning method to be evaluated. The positioning precision was defined as the mode distance between each estimate and the centroid of the estimates because the distribution form of the distance was right-skewed. The mean±standard deviation (sd) and 95-percentile distances of each estimate from the centroid of the estimates were also calculated. Furthermore, the directional distribution (95% deviational ellipse) of the estimates was displayed for visual comprehension. The probability of location was defined as the proportion (%) of the number of estimates to the number of actual transmissions made by each Gold code transmitter. The positional accuracy (i.e. the distance between the estimated and actual location) was not evaluated in this study because the predicted positional precision was significantly smaller than the GPS accuracy (normally a few metres).

A generalised linear mixed model (GLMM) using the gamma distribution with the log link function was employed to examine whether the location of the transmitter (near the triangulation centre, near the vertex, on the baseline, and outside triangulation) affected positional precision. The model included the location of the transmitter as a fixed effects factor and the transmitter IDs as a random effects factor. The model was fitted using the glmer function in the lme4 package for R ver. 3.1.3 (R Core Team, 2017).

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**Table 3-1.** Summary of deployment information for five transmitters employed during stationary testing.

| Transmitter ID | Location | Installation depth (m) |
|:---:|:---:|:---:|
| T1 | Near triangulation Centre | 2.5 |
| T2 | Near vertex | 2.5 |
| T3 | On baseline | 2.7 |
| T4 | Near triangulation Centre | 2.5 |
| T5 | Outside triangulation | 2.8 |

## 3.2.5 Free-swimming fish test

Positioning performance was also tested using Siebold's wrasse as free-swimming fish specimens. The Siebold's wrasse is one of the most common species in rocky areas on the western coast of Japan (Mabuchi and Nakabo, 1997), making it is easy to obtain multiple specimens from the same location. Furthermore, male Siebold's wrasses demonstrate site fidelity during the spawning season (from late September to mid-November), which occurred during the experimental period (Matsumoto et al., 1997). These wrasses were well suited for studying their movements using the developed positioning system as they travelled in their natural environment from an unsuitable to a suitable place (rocky areas) after displacement.

Seven male Siebold's wrasses [N=7, total length (TL): 21.5±0.9 cm (mean±sd), body weight (BW): 150.1±15.2 g] (Table 3-2) were captured by fishing with a baited hook and line in two rocky areas (Fig. 3-1c) between 23 to 26 October 2015. One of the seven fish (F1) was caught in rocky area 1, and the remaining six (F2−7) in rocky area 2. Specimens were kept in a round tank (~1280 mm diameter, 815 mm height, 1000 litre volume) for 2–5 days until the experiment. The tank was filled with ~600 litre fresh seawater flowing of ~10 litre·min$^{-1}$. Gold code transmitters (AQPX-1040PT, AquaSound Inc., Kobe, Japan) with signal transmitting intervals of ~5.0 s were surgically inserted into the abdominal cavities of the fish under

anaesthesia induced with 0.1% 2-phenoxyethanol. The fish were placed between rubber sponges in baths of fresh bubbling seawater throughout the procedure. After the procedure, they were kept in fresh seawater until they came out of the anaesthesia.

**Table 3-2.** Fish information of the seven tagged Siebold's wrasses.

| Fish ID | Body weight (g) | Total length (cm) |
|---------|-----------------|-------------------|
| F1 | 173 | 22.4 |
| F2 | 152 | 21.5 |
| F3 | 135 | 21.1 |
| F4 | 127 | 20.0 |
| F5 | 152 | 22.3 |
| F6 | 159 | 22.2 |
| F7 | 153 | 21.1 |
| Mean ± sd | 150.1 ± 15.2 | 21.5 ± 0.9 |

The tagged fish were then placed in an upside down transparent container with small holes and kept on the sea bottom (3.5 m depth) at a release point inside the array approximately 87 m and 132 m away from rocky areas 1 and 2 (Fig. 3-1c), respectively. The release point was on a flat sea bottom of muddy sediment. As the tagged fish inhabited and had been caught in rocky areas, the release point was suspected to be an unsuitable environment for them. After 35 min of acclimation, the container was slowly opened and the tagged fish were simultaneously released at 14:56 on 28 October, 2015. Their 3D positions were monitored until 9:00 on 30 October. Whether the tagged fish homed to the rocky area was determined using an additional receiver deployed at each rocky area the next day. The additional two receivers were not used for positioning.

Outliers were removed from raw data obtained by the proposed positioning method using speed limitation. I conservatively set 5 TL・s$^{-1}$ as

the maximum swimming speed in this study due to the cruising speed [generally less than 2–3 body length (BL)・s$^{-1}$]. Burst swimming was not considered because, in this study, the target movement was relatively long-term movement while traveling. If the speed between two consecutive raw data points exceeded 5 TL・s$^{-1}$, the latter point was removed. Removed data comprised 6.8±8.8% of the raw data (N=7, range: 0–19.5% or 0–335 data points). To test the positioning performance of the proposed method for locating free-swimming fish following the same method as for the stationary test, the probabilities of location and positioning intervals of the seven tagged fish within the array were examined. The positioning interval was defined as the mean value of intervals between all consecutive pairs of data points.

To examine the intermittent locomotion of fish travelling from an unsuitable to a suitable area, their locomotion modes were categorised into two types: 'stop' and 'move'. First, routes between the release point and rocky areas were extracted using a swimming depth threshold of <4 m. Movements at night [30 min after sunset (17:52) to 30 min before sunrise (5:57)] were omitted from further analyses because Siebold's wrasse follow the diurnal habits common to labrid fish and do not travel distances at night (e.g. Nishi, 1989; 1990). Route data was spatially interpolated every 5 s if there were zero or one missing values between each data step (a temporal interval between two consecutive data points <~10 s). If the distance between two consecutive estimates was ≤0.64 m, the tagged fish was categorized in the 'stop' mode at both points. A 'stop' phase was defined as sequential 'stop' modes (Fig. 3-3). Conversely, if the distance was >0.64 m, the tagged fish was categorized in the 'move' mode at the latter point. A 'move' phase was defined as sequential 'move' modes (Fig 3-3). Missing values were ignored in distance calculations. The 0.64 m threshold was defined as 2× the maximum 95-percentile distance from the stationary test (0.32 m; see Table 3-3 in results section). Turn angles in the two phases were then calculated. The turn angle in 'move' phases was defined as the difference in destinations between any set of three sequential estimates

(-180°<θ≤180°; see Fig. 3-3) whereas the turn angle of 'stop' phases was defined as the difference between the final destination of the preceding 'move' phase and the first destination of the following 'move' phase (-180°<θ≤180°; see Fig. 3-3). Turn angles in 'stop' phases were categorised into two types, first-half turns and second-half turns, according to our hypothesis that the tagged fish would display movement with angled turns during the first-half of their return due to tendencies of homing fish displaying searching behaviour just after release (e.g. Mitamura et al., 2012 and Calson et al., 1995). If the tagged fish stayed overnight in their travels, movements until 30 min after sunset and from 30 min before sunrise were categorised into the first-half and the second-half, respectively. If the tagged fish finished traveling within the release day, the first and second halves were separated by the traveling period. Turn angles were calculated only when all time step data was available without missing values. Circular statistical analyses were performed using functions in the circular package for R ver. 3.1.3 (R Core Team, 2017).

To show the spatiotemporal resolution of the proposed method, the number of 'stop' phases, distance travelled, speed, and turn angles were compared between five resampled data sets. Routes were also interpolated at 10, 30, 60, and 300 s intervals using the manner previously described. The distance travelled was calculated as the cumulative distance from 'move' phases and the distance between first and last point in 'stop' phases. Speed was calculated only where there were no missing values between any two consecutive interpolated data points in the 'move' phase. The speed distribution in each sampling interval (5, 10, 30, 60, and 300 s) was estimated using a kernel density estimation. Turn angles during the 'move' phase, first half of the 'stop' phase, and second half of the 'stop' phase at each sampling interval were examined using a Rayleigh test (Zar, 2010) to determine whether there was a uniform distribution. If there was not a uniform distribution, a V-test (Zar, 2010) was conducted to determine whether it was concentrated around 0°.
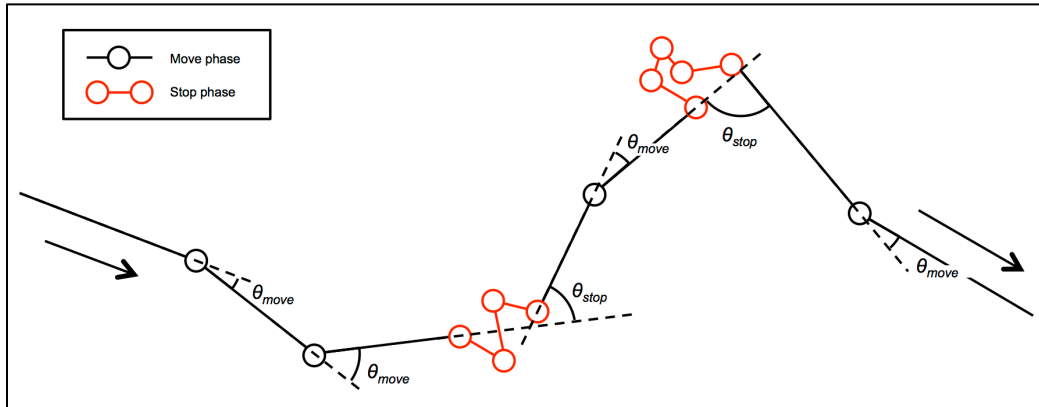
**Fig 3-3.** Schematic diagram of turn angles in a 'move' phase (θmove) and 'stop' phase (θstop).

## 3.3 Results

### 3.3.1 Stationary test

The positional precision was 0.056±0.033 m (N=5; range 0.012−0.093 m; Fig. 3-4, Fig. 3-5, and Table 3-3) and probability of location was 83.6±14.0% (N=5, range 60.5−96.0%; Table 3-3). The estimated distribution of the distance between each estimate and the centroid of estimates was right-skewed in all transmitters (Fig. 3-4). The 95-percentile of distance was 0.232±0.070 m (N=5, range 0.136−0.322 m; Fig. 3-4 and Table 3-3). The pressure sensor in the transmitters provided accurate depth information within a strict range, enabling high spatial resolution 3D positioning (Table 3-1, and Table 3-3). The results from the GLMM indicated that the positional precision of transmitters installed near centroid was higher than that of transmitters installed at other places (Table 3-4).

**Table 3-3.** Summary of the positioning results of the five transmitters employed during stationary testing.

| Transmitter ID | Depth provided by pressure censor (m) | Detection rate (%) | Probability of location (%) | Distance (m) each estimate − centroid of estimates | | |
|---|---|---|---|---|---|---|
| | | | | Mode | Mean ± sd | 95% |
| T1 | 2.5 ± 0.02 | 95.7 ± 5.0 | 96.0 | 0.012 | 0.081 ± 0.049 | 0.20 |
| T2 | 2.5 ± 0.06 | 87.8 ± 14.9 | 80.6 | 0.093 | 0.111 ± 0.058 | 0.25 |
| T3 | 2.7 ± 0.05 | 79.0 ± 18.5 | 60.5 | 0.037 | 0.110 ± 0.081 | 0.32 |
| T4 | 2.5 ± 0.05 | 92.6 ± 10.9 | 90.7 | 0.057 | 0.069 ± 0.027 | 0.14 |
| T5 | 2.8 ± 0.06 | 90.8 ± 8.9 | 90.4 | 0.080 | 0.115 ± 0.063 | 0.26 |

**Table 3-4.** Generalised linear mixed model for positional precision. Model coefficients for fixed effects are presented.

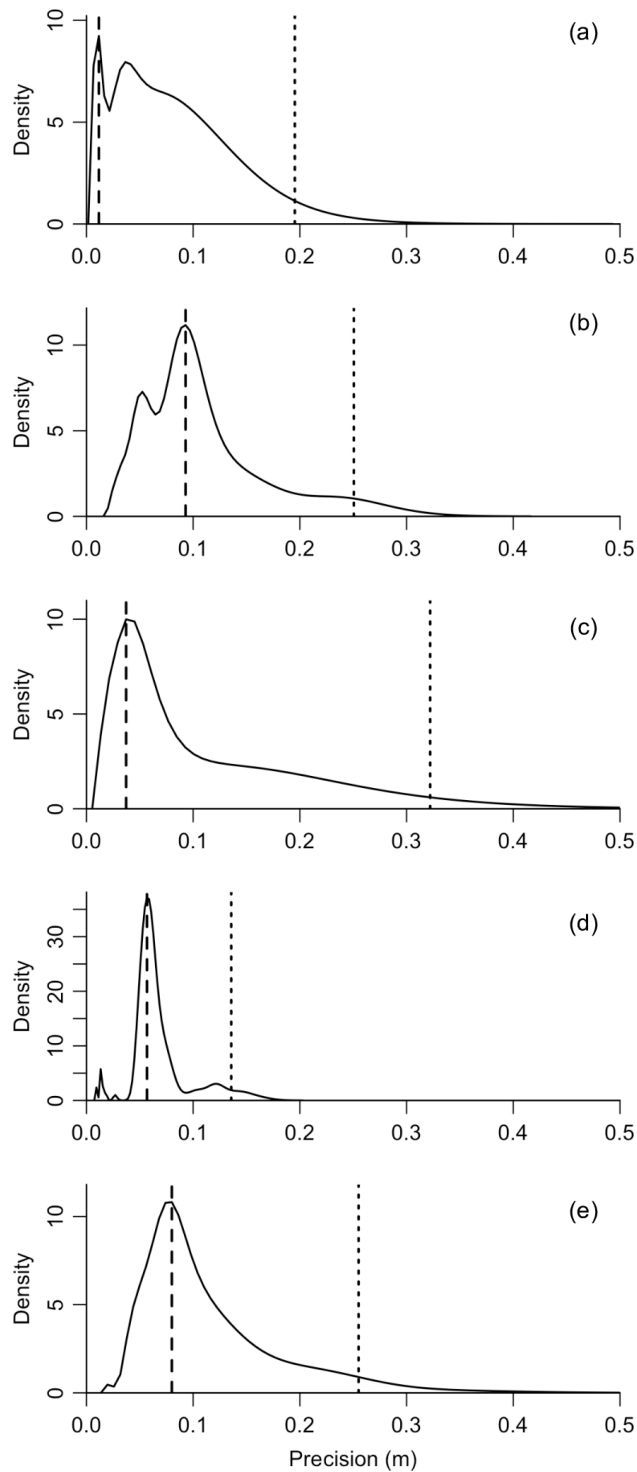| Explanatory variables | Coefficients | Standard error | t-value | p-value |
|---|---|---|---|---|
| (Intercept) | -2.59014 | 0.02654 | -97.577 | <0.001 |
| Near centroid* | | | | |
| On baseline | 0.38023 | 0.05499 | 6.915 | <0.001 |
| Outside triangulation | 0.42760 | 0.04702 | 9.093 | <0.001 |
| Near vertex | 0.38738 | 0.04812 | 8.051 | <0.001 |

* Standard category

**Fig. 3-4.** Kernel density estimation of the distance between the centroid of all estimates and each estimate of the five stationary transmitters. The distribution of transmitters 1–5 is represented in (a)–(e), respectively. The dashed line indicates the mode value, and the dotted line indicates the 95-percentile value.
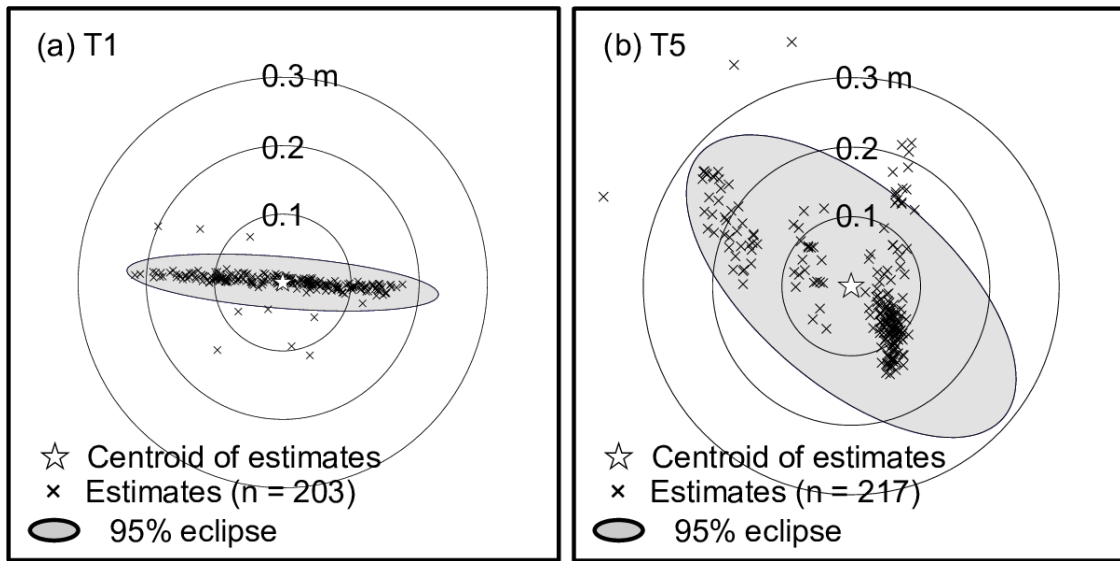
**Fig. 3-5.** Stationary test results. (a) 2D estimates with their centroid, and 95% eclipse for the deployment of T1 near a centroid of triangulation, and (b) T5 outside triangulation.

## 3.3.2 Free-swimming fish test

The 3D positions of the seven tagged Siebold's wrasses were obtained simultaneously with fine temporal resolution as they escaped from an unsuitable to a suitable area by freely swimming around the receiver array after release (Fig 3-6). The tagged fish stayed within the 13590.19 m² array for 7574.3±17419.3 s (N=7, range 280.1−47023.9 s; see Table 3-5), during which 1071±2424 estimates (N=7, range 27−6558 estimates; see Table 3-5) were obtained. Their probability of location was 69.1±15.3% (N=7, range 46.6−89.4%; see Table 3-5), and the positioning interval was 7.4±1.7 s (N=7, range 5.6−10.4 s; see Table 3-5). Their swimming depth within the array averaged ~3 m, suggesting they remained on the seafloor during both 'stop' and 'move' phases at least in the shallower zone of the study area (Table 3-5 and Fig 3-6).
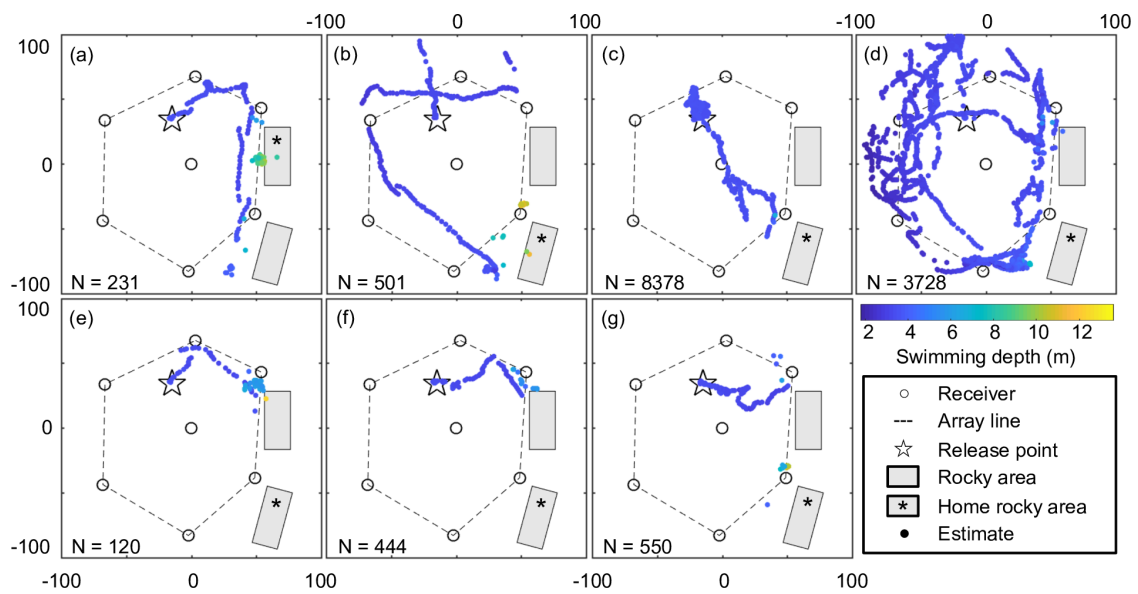
**Fig 3-6.** 3D estimates of seven tagged Siebold's wrasses (F1−7) during the monitoring period. Estimates for F1−7 are shown in (a)−(g), respectively, with colour variations indicating swimming depths.

**Table 3-5.** Positioning results within the array of the seven tagged Siebold's wrasses.

| Fish ID | Residence time (s) | N of estimates | Probability of location (%) | Mean positioning interval (s) | Swimming depth (m)* |
|---------|--------------------|----------------|-----------------------------|-------------------------------|---------------------|
| F1 | 461.4 | 58 | 59.8 | 8 | 3.5 ± 0.1 |
| F2 | 521.9 | 93 | 89.4 | 5.6 | 3.4 ± 0.1 |
| F3 | 47023.9 | 6558 | 69.2 | 7.2 | 3.6 ± 0.1 |
| F4 | 280.1 | 27 | 46.6 | 10.4 | 3.4 ± 0.1 |
| F5 | 320.1 | 38 | 57.6 | 8.4 | 3.4 ± 0.1 |
| F6 | 1690.1 | 270 | 79.9 | 6.3 | 3.5 ± 0.1 |
| F7 | 2723 | 458 | 81.2 | 5.9 | 3.3 ± 0.1 |
| Mean ± sd | 7574.3 ± 17419.3 | 1071 ± 2424 | 69.1 ± 15.3 | 7.4 ± 1.7 | - |

[a] Represented as mean±standard deviation

All tagged fish returned to suitable areas (original rocky areas) from the unsuitable release area (a muddy flat area) before the following morning. Four of the seven tagged fish (F1, F5, F6, and F7) moved east and settled around the rocky areas before sunset on the release day, and a single tagged fish (F1) homed to its original location (Figs 3-6a, 3-6e, 3-6f, and 3-6g). One tagged fish (F2) moved toward the north immediately following release, then turned clockwise and reached home before sunset by traveling around the array in a 'Z' pattern (Fig. 3-6b). The remaining two tagged fish (F3 and F4) stayed around the array overnight: one (F3) settled near the release point, whereas the other (F4) settled at a northern point in the array after wandering the northern and western sides of the array until sunset. The next morning after sunrise, F3 and F4 moved toward the rocky areas, and F3 homed to its original location (Figs 3-6c and 3-6d).

Intermittent stop-and-go locomotion was observed in the routes of the seven tagged fish. Using 5 s sampling intervals, the tagged fish stopped 11.3±10.3 times (N=7, range 1−30 times) on routes lasting 17851.4±27545.6 s (N=7, range 275−58545 s; Table 3-6) excluding nights. The duration of the 'stop' phase was 277.6±653.5 s and the median duration was 30.0 s (N=79, range 5−3555 s). The 'move' phase turn angle distribution at 5 s sampling intervals was significantly concentrated around 0° (V-test, mean vector=0°, $W$=0.72, p<0.001; Table 3-6). The first half 'stop' phase distribution was not significantly concentrated in any direction (Rayleigh test, $W$=0.14, p>0.05; Table 3-7); conversely, the second half distribution was significantly concentrated around 0° (V-test, mean vector=0°, $W$=0.61, p<0.001; Table 3-7).

Increasing the sampling interval decreased the number of stop phases (Fig. 3-7a) and also decreased the distance travelled (Table 3-6 and Fig. 3-7b). The speed distribution in the 'move' phase became gradually right-skewed when increasing the sampling interval (Fig. 3-7c). The turn angle distribution of the 'move' phase was significantly concentrated around 0° at all six sampling intervals (V-test, mean vector = 0°; see Table 3-7), while that of the first half of the 'stop' phase was not significantly

concentrated in any direction with all six sampling intervals (Rayleigh test, p>0.05; see Table 3-7). In the second half of the 'stop' phase, there was no significant concentration in any direction in the turn angles sampled at 10, 30, and 60 s intervals (Rayleigh test, p>0.05; see Table 3-7) although there was less data with increased sampling intervals. At 5 s intervals, the turn angle distribution in the second half of the 'stop' phase had significant concentration around 0° (V-test, mean vector = 0°, $W$=0.61, p<0.001; see Table 3-7).

**Table 3-6.** Duration and distance travelled in five resampling intervals of movement routes of seven tagged Siebold's wrasses.

| Fish ID | Duration[b] (s) | Distance travelled (m) | | | | |
|---|---|---|---|---|---|---|
| | | 5 s | 10 s | 30 s | 60 s | 300 s |
| F1 | 825 | 229.8 | 219.7 | 198.7 | 163.7 | 60.4 |
| F2 | 3050 | 494.4 | 492.2 | 469.8 | 455.0 | 407.8 |
| F3[a] | 15045 | 288.6 | 279.3 | 251.1 | 222.6 | 146.2 |
| F4[a] | 14250 | 1817.5 | 1808.4 | 1759.6 | 1666.5 | 1188.5 |
| F5 | 275 | 100.6 | 98.0 | 86.2 | 78.5 | n/a |
| F6 | 1795 | 106.9 | 100.6 | 89.6 | 68.9 | 16.9 |
| F7 | 2720 | 117.7 | 116.4 | 96.7 | 87.8 | 62.7 |

[a] Without nights (17:52−5:57 the next day).

[b] In 5 s resampling interval.

**Table 3-7.** Summary of turn angles in 'move' mode, and first and second halves of 'stop' mode at six sampling intervals**.**

| Phase | Sampling interval (s) | Number of data | Test statistics[a] | p-value |
|---|---|---|---|---|
| Move | 5 | 1373 | V, $W$=0.72 | <0.001 |
| | 10 | 1120 | V, $W$=0.50 | <0.001 |
| | 30 | 567 | V, $W$=0.34 | <0.001 |
| | 60 | 381 | V, $W$=0.28 | <0.001 |
| | 300 | 179 | V, $W$=0.22 | <0.001 |
| Stop in first half | 5 | 57 | R, $W$=0.14 | >0.05 |
| | 10 | 47 | R, $W$=0.29 | >0.05 |
| | 30 | 29 | R, $W$=0.06 | >0.05 |
| | 60 | 21 | R, $W$=0.12 | >0.05 |
| | 300 | 17 | R, $W$=0.36 | >0.05 |
| Stop in second half | 5 | 24 | V, $W$=0.61 | <0.001 |
| | 10 | 19 | R, $W$=0.14 | >0.05 |
| | 30 | 8 | R, $W$=0.55 | >0.05 |
| | 60 | 6 | R, $W$=0.16 | >0.05 |
| | 300 | 2 | n/a | -- |

[a] n/a indicates that there were too few data points to test. The V-test (V) with a mean vector of 0˚ was used when a null hypothesis of uniform distribution was rejected by the Rayleigh test (R).
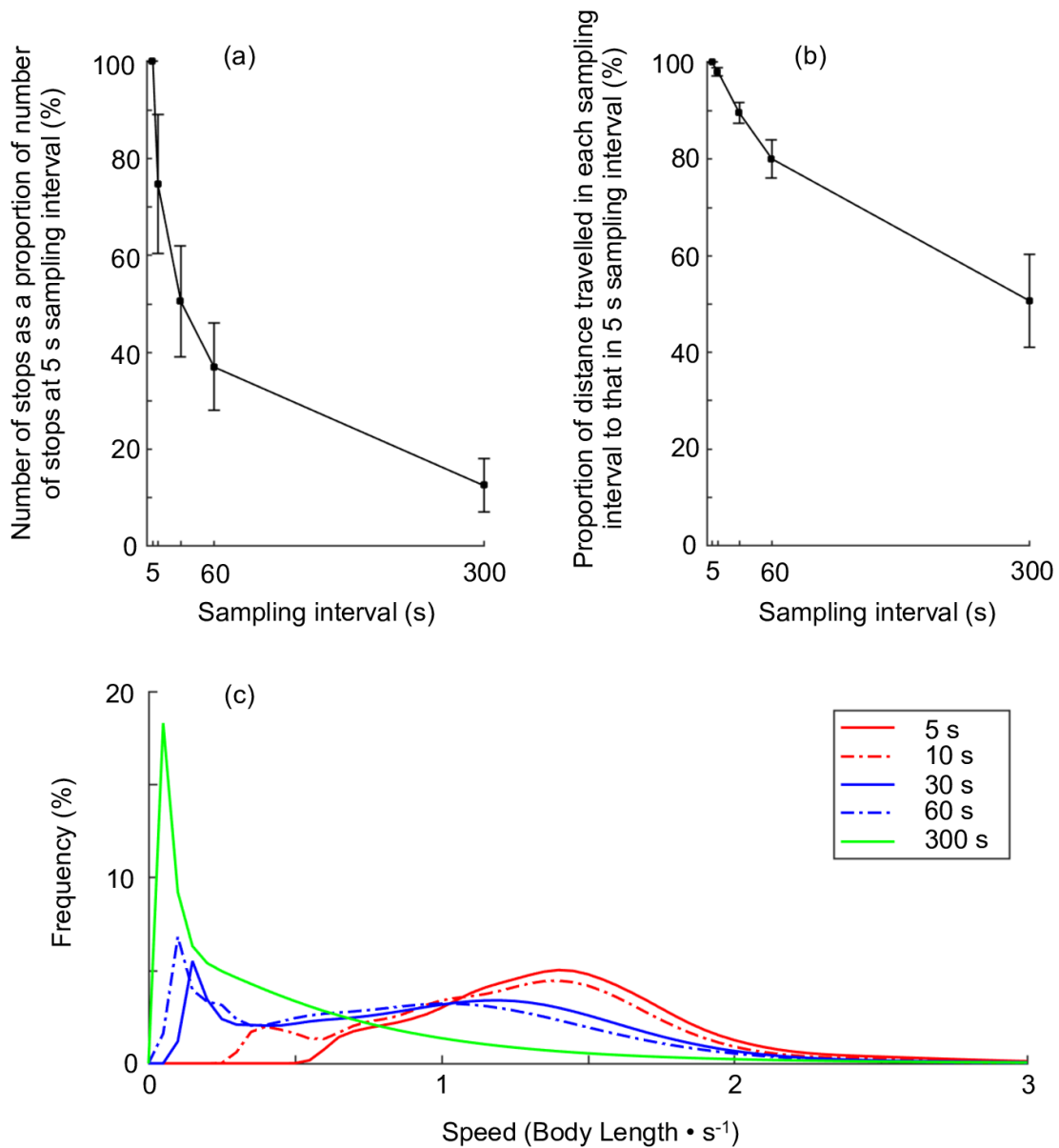
**Fig 3-7.** Comparison of stop numbers, distance travelled, and speed of tagged Siebold's wrasse. (a) Number of 'stop' phases and (b) distance travelled as a proportion of that at 5 s sampling intervals for the seven tagged Siebold's wrasse in five resampling interval lengths (5, 10, 30, 60 and 300 s). The error bar indicates the standard mean error. (c) Kernel density estimation of movement speed only during 'move' phase in the five resampling interval lengths. The speed was standardised (divided by the total length of each fish).

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## 3.4 Discussion

### 3.4.1 Stationary test

The proposed positioning system using Gold code transmitters and receivers positioned multiple stationary transmitters simultaneously and in 3D with high-precision spatial resolution (<10 cm) and a high probability of location (average >80%). The results demonstrate that the system is capable of simultaneously observing the movements of multiple aquatic animals at a high spatiotemporal resolution. The results from the GLMM show that the positional precision near the centroid of triangulation was significantly higher than that of other installation sites (near the vertex, on the baseline, and outside triangulation) although fewer transmitters were deployed during the stationary test (Table 3). This result is generally known and has been demonstrated in previous studies of positioning methods using the TDOA concept (see Cote et al., 1998; Klimley et al., 2001; Andrews et al., 2011; Espinoza et al., 2011; Gergé et al., 2012; Roy et al., 2014). However, the positional precision of five installation sites was considerably high. Even at the 95% point, the distance from the centroid of estimates was only 0.322 m (Table 1 and Fig 4). One transmitter (T3) had a relatively lower probability of location than those of the remaining four transmitters. This may have been caused by lower detection rates directly linked to poor mutual detection in three receivers (Table 1). Multiple non-detection and/or false-alarm factors should be considered (Kessel et al., 2014): differences in bathymetry or obstacles between transmitters and receivers (see Welsh et al., 2012); environmental noise from motors, snapping shrimp, and other taxa (see Simpfendorfer et al., 2008); and sea surface conditions (see Gjelland and Hedger, 2013). Although signal collisions influenced detection rates (Voegeli et al., 1998), signals in the presented biotelemetry system rarely collided due to their short (2 ms) pulse duration (Miyamoto et al., 2011). This is one of the major advantages of the developed system. The lower detection rate may also be explained by the differences between the transmitters and/or the installation sites. The ultimate cause for this lower

detection rate, however, remains unidentified.

Receiver array geometry and other environmental factors can decrease detection rates (Andrews et al., 2011) and affect positioning performance. Furthermore, biotelemetry has been performed in various fields such as shallow seas, deep seas, narrow rivers or channels, small ponds, and large lakes (Hussey et al., 2015) where various environmental factors affect the propagation of ultrasonic waves (Kessel et al., 2014), resulting in the deterioration of positional precision and location probability. Receivers were deployed to form multiple equilateral triangles, and only equilateral triangulations were used for positioning with the proposed method. Acute- or obtuse-angled triangulation can mathematically deteriorate positional precision due to the use of hyperbolic curves during TDOA positioning (Espinoza et al., 2011). It is necessary to deploy receiver arrays that are mathematically suitable for calculating as many locations as possible. The influence of environmental factors in the proposed biotelemetry system shall be investigated in future studies.

## 3.4.2 Free-swimming fish test

Seven tagged Siebold's wrasses were simultaneously positioned at high temporal resolution, providing precise data on their movement routes and behavioural intermittence. Intermittent stop-and-go locomotion was observed as the fish travelled from an unsuitable to a suitable area after their displacement. To the authors' knowledge, this study was the first report to demonstrate the homing ability of Siebold's wrasse. At a sampling interval of 5 s, turn angles in the 'move' and the second half of the 'stop' phase were significantly concentrated at 0°; conversely, those in the first half of the 'stop' phase were not concentrated in any direction (Table 5). This implies that the tagged fish tended to change direction after stopping with relatively straight movements during the first half, and travelled in a relatively straight direction during the second half. Thus, the stop-and-go behavioural intermittence of Siebold's wrasse observed in this study

appeared linked to reorientation during the first half of traveling from an unsuitable to a suitable area. Animal movements can be understood as scanning and reorientation sequences such as the saltatory search (O'brien et al., 1990) or Lévy patterns (Bartumeus, 2007; Bartumeus and Levin, 2008). Therefore, the tagged fish may have searched for the direction leading to a suitable place (a rocky area, as their familiar location), by scanning for environmental cues using sensory organs. It is possible the tagged fish used an olfactory cue from their habitat, similar to salmonids (Ueda et al., 1998; Kitahasih et al., 2000). For instance, black rockfish *Sebastes cheni* perform back-and-forth movements in the direction of the current (intermittent locomotion with 180° turns) to detect their homeward direction using olfactory cues (Mitamura et al., 2012). The tagged fish may have sensed an olfactory cue related to the current direction, for instance, an odour of conspecific females from their original location due to the spawning season. They may have also sensed other cues such as bathymetric gradients, including changes in light, temperature, and pressure with depth (see Meckley et al., 2017). Although it is ultimately unknown what the tagged fish did during their first half 'stop' phases, this study demonstrated that the proposed positioning method is capable of observing the intermittent locomotion of free-swimming fish in their natural environment.

It was confirmed that the proposed temporally fine-scale positioning method enables observations of the stop-and-go travels undertaken by tagged fish. The number of observed 'stop' phases decreased as the resampling interval increased (Fig 3-7a). For example, relative to the original 5 s sampling interval, only 75% of 'stop' phases were observed at 10 s intervals. Although the same result might have obtained were the sampling interval less than 5 s, the results show that there were numerous undetected 'stop' phases when longer intervals were used. However, at a sampling interval of 5 s, it was observed that the tagged fish tended to reorient after stopping with straight movements during the first half, and tended to travel in a straight direction thereafter during the second half.

This was not observed at >10 s sampling intervals. It was thus the positioning method, with fine-scale temporal resolution, that enabled the observation of stop-and-go travels of tagged fish and the elucidation of their straight movements and reorientation after stopping. Furthermore, the distance travelled was underestimated as resampling intervals increased (Fig 3-7b). Although the distance travelled tends to be overestimated at high frequency sampling intervals due to the fine scale of resolution, especially with GPS (Ryan et al., 2004), it is suspected that the distance travelled at 5 s sampling intervals was not significantly overestimated because the step length while stopped was not considered, and this often leads to overestimations. The speed during 'move' phases was also underestimated as the sampling interval lengthened (Fig 3-7c). It should be considered that a precise sampling interval can never perfectly describe whole movements of tagged fish; nevertheless, it was the case that 'stop' phases related to reorientations were observed at precise (i.e. 5 s) sampling intervals, and not observed at >10 s sampling intervals.

Within the array, the probability of location of the tagged fish was 69.1±15.3%, which was relatively low compared to that of the stationary test (83.6±14.0%). The positioning interval, however, was 7.4±1.7 s, giving an estimate of tagged fish locations at least once every ~10 s. Conventional biotelemetry systems typically set their signal transmitting interval between 30–120 s to minimize collisions (see Pincock, 2008); for example, during the simultaneous observation of moving routes using a conventional biotelemetry system, Mitamura et al. obtained positional data sets of the homing behaviour of four black rockfish (*Sebastes cheni*) at time intervals of 60 s or greater (Mitamura et al., 2012). CDMA MAP technology, which has simultaneous positioning capabilities, was able to localise ~75% of transmissions at 15 s intervals, such that there was an estimate every 20 s on average (Coke et al., 2005). Compared to these positional intervals, the proposed positioning method has the advantage of frequent monitoring in movement routes using positional data. Moreover, the biotelemetry system used in this study could potentially provide more temporally precise

movement observations if the signal-transmitting interval were shortened (i.e. 1.0 or 2.0 s); however, the interval was set to ~5.0 s in this experiment. The utilized biotelemetry system is capable of simultaneously identifying multiple high-temporal resolution (<2 s) signals transmitted at 1.28 s intervals (showed in Chapter 2), such that positions can be obtained at a higher temporal resolution than 10 s. These temporally precise positions can provide more detailed movement data than the results shown in this study.

This study developed a positioning method capable of simultaneously pinpointing multiple fish with fine-scale data both in space and time using a new telemetry system that was validated by providing precise 3D positions of seven Siebold's wrasses. Those spatiotemporally fine-scale positions allowed intermittent locomotion to be observed, and revealed that the tagged Siebold's wrasses tended to reorient after 'stop' phases during the first half of their voyage. This method will provide insights into unknown aquatic animal behaviour by providing more detailed movement observations in the future.

## 3.5 Conclusions

In this chapter, a 3-dimensional acoustic positioning method which can simultaneously localise multiple transmitters at spatially and temporally fine-scale was developed and applied to observing homing behaviour of a coastal fish species, Siebold's wrasse. It was demonstrated that the developed positioning method could observe detailed behaviour of the fish simultaneously bot at high frequent interval and at high positioning precision. This positioning method can be adequately applied to observe movement behaviour of schooling fish. In the next chapter, a challenge to observe schooling behaviour of homing fish, black rockfish, is described.

# Chapter 4

# Observation of homing behaviour of black rockfish released in a group: do black rockfish use social cues in homing?

## 4.1 Introduction

Organisms live in groups that occupy various spatial and temporal scales. Living in groups provides various advantages (Krause and Ruxton, 2002; Sumpter, 2010) like increasing food search efficiency (Pitcher et al., 1982), reducing predation risk (Partridge, 1982; Magurran, 1990), and saving locomotion energy (Weihs, 1973; Marras et al., 2015; Portugal et al., 2014; Hemelrijk et al., 2015). Enhancement of navigational ability is also one of the advantages of group movement. For example, pigeons home more efficiently when they travel in flocks than when they fly alone (Biro et al., 2006; Dell'Ariccia et al., 2008). King penguin chicks return to their places of origin more efficiently when they travel in pairs than when they move alone (Nesterova et al., 2014). The 'many-wrongs principle' (Bergman and Donner, 1964; Simons, 2004; Codling et al., 2007) proposes that collective movement averages out individual errors and improves overall navigational ability.

Homing behaviour is correlated with navigational ability. It has been well documented in anadromous and catadromous fishes like salmonids (Ueda, 2012) and eels (Baltazar-Soares and Eizaguirre, 2017). However, most of the studies investigating collective movement and navigational ability have focussed on terrestrial animals. Research on the collective navigation in schooling fish is scarce. Collective behaviour in homing fish is well known. Collective navigation in salmonids has been hypothesised and explored (Berdahl et al., 2014). Nevertheless, that study was based on annual population data rather than empirically acquired field movement data as was the case with terrestrial animals.

Schooling fish can have very wide migration ranges. Therefore, it may be very difficult to investigate their collective navigation. Salmonids and eels perform trans-oceanic navigation from natal river or marine areas. These migration routes are too vast to be observed in their entirety. To investigate collective navigation in fish, it is necessary to define a target individual and observe its movement within a limited area such as short-range homing.

Rockfish of the genus *Sebastes* are known for their strong site fidelity and homing ability after artificial displacement (e.g., Green and Starr, 2011; Matthews, 1990a; Mitamura et al., 2002, 2005, 2009, 2012; Reynolds et al. 2010). The black rockfish *S. cheni* is one of the most common and commercially important *Sebastes* species in Japan. It distributes along the coast of southern Hokkaido southward to Kyushu (Harada, 1962). Adult black rockfish inhabit rocky areas and have small home ranges (Harada, 1962; Mitamura et al. 2009). This species can navigate back to its original habitat even after artificial displacements of 1–4 km (Mitamura et al., 2002). They predominantly use olfaction to return home from unfamiliar areas (Mitamura et al. 2005; 2012). However, they also use vision in familiar areas (Mitamura et al. 2012). Juvenile black rockfish form large schools whereas the adults tend to be solitary and seldom form groups (Harada, 1962). Their preferred habitats for aggregation are rocky areas. However, there is no clear evidence as to whether they move in schools. External

factors (biotic and abiotic), internal state, motion capacity, and navigational capacity all influence movement (Nathan et al., 2008). The presence of conspecifics can influence the movement of individuals (Delgado et al., 2014). In a preliminary study, several black rockfish started to move with 1–4 other individuals after 16 were simultaneously released (Takagi, personal observation; Fig. 4-1). If black rockfish recognise other conspecifics by vision and other senses, then their movements could be at least partially motivated by other conspecifics. Therefore, adult black rockfish released in a group could move together using social cues from other conspecifics in their homing journey.
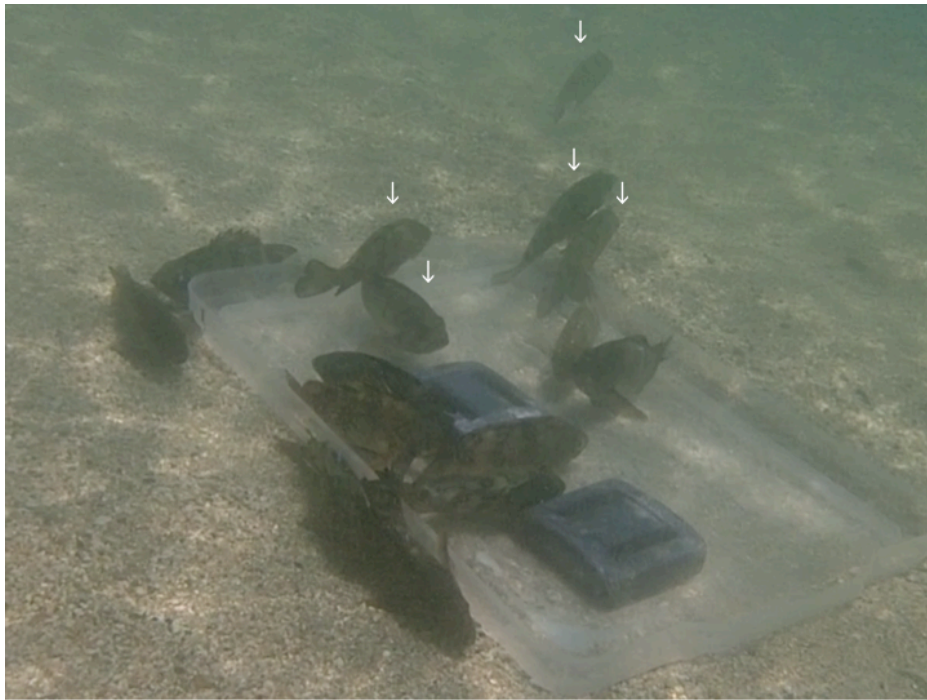


**Fig. 4-1.** Black rockfish *Sebastes cheni* released simultaneously and started to move with other individuals. Five arrowed individuals left together.

Here, I propose two hypotheses: (1) black rockfish released with other individuals return to their original habitat by forming a group after artificial displacement, and (2) group homing improve spatial and temporal migratory efficiency compared with individual homing because of collective intelligence. My first objective was to test those two hypotheses. My second was to determine how black rockfish return to their place of origin. I observed the trajectories of homing black rockfish using fine-scale 3D acoustic positioning developed in the previous chapter.

## 4.2 Materials and Methods

The Governor of Hiroshima Prefecture issued the permit to collect fish around Ikuno Island for the purposes of this study in this chapter. All procedures including the sampling protocol and the tagging surgery were approved by the Animal Research Committee of Kyoto University (Permit No. Inf-K17004).

### 4.2.1 Study area and deployed array

Field experiments were conducted in July 2017 in a shallow sea area near Ikuno Island (34°29'N, 132°92'E) in the Seto Inland Sea, Japan (Fig. 4-2). The actual study area was on the east coast of Ikuno Island. The tidal difference during the experiments was ~3.5 m. Fourteen receivers (AQRM-1000, AquaSound Inc., Kobe, Japan; 64 mm diameter × 300 mm length) with overlapping detection ranges were deployed on the sea bottom of the study area (Fig. 4-2). To synchronise the internal clocks of the receivers, one gold code transmitter (AQTD-600P, AquaSound Inc., Kobe, Japan; 27 mm diameter × 130 mm length; sound pressure level 160 dB re 1 µPa at 1 m; transmitting interval 60 s) was placed on every receiver except one on the west side of the floating pier as shown in Fig. 4-2.

Current direction and speed were measured with an electromagnetic current meter (INFINITY-EM, JFE-Advantech, Kobe, Japan; direction

range, 0–360°; resolution, 0.01°; accuracy, ± 2°; speed resolution, 0.02 cm s$^{-1}$; speed accuracy, ± 1 cm s$^{-1}$ or ± 2%). The burst mode of the current meter was used to record the current. The current meter was programmed to record a single datum every 3 min at an average of 30 successive data points at 1-s intervals. The current meter was deployed at two points on the sea bottom. One of these was inside the bay near the release point and the other was located outside the bay through which the tagged fish passed in their homing journey (Fig 4-2).
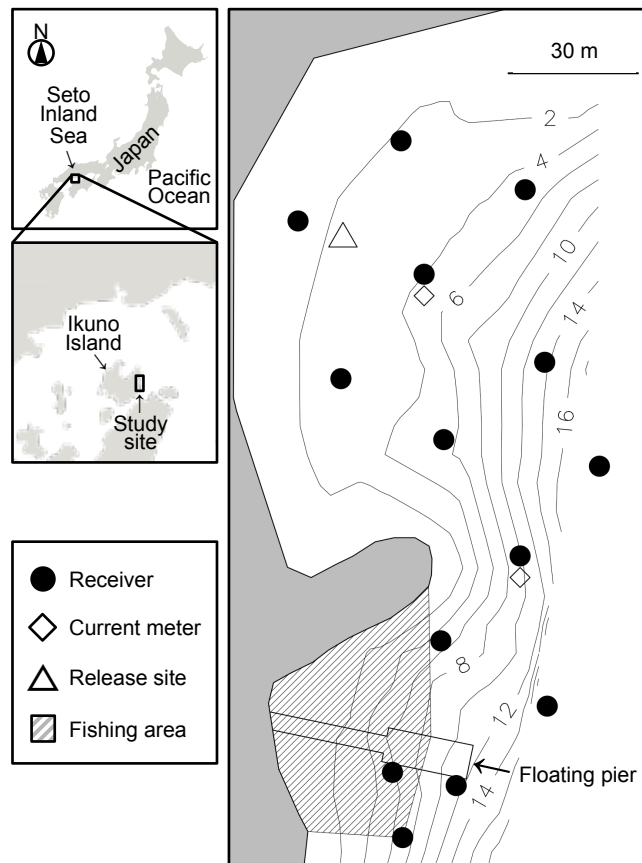


**Fig. 4-2.** Acoustic array of 14 receivers deployed off Ikuno Island, central Seto Inland Sea, southwestern Japan. Two current meters were deployed inside and outside the bay. The black rockfish used in this study were caught in the fishing area around the floating pier. Lines along the coastline indicate depth contours.

## 4.2.2 Tagging and releasing fish

Twelve black rockfish [N=12, total length (TL): 21.2±1.3 cm (mean±sd), body weight (BW): 154.1±30.5 g; Table 4-1] were captured between 13 May 2017 and 1 July 2017 using baited hooks and lines around the floating pier (Fig 4-2). It was difficult to collect more than a few black rockfish at one time in this study area. Fishing positions relative to the floating pier were recorded. The rockfish were labelled with dart tags (each ~30 mm in length) for identification purposes. Specimens were kept in a round tank (~1,280 mm diameter, 815 mm height, 1,000 L volume) for 2–50 d until the experiment started. The tank was filled with ~600 L fresh seawater flowing at a rate of ~10 L min$^{-1}$. Black rockfish exhibited the ability to home to their original habitats several months after capture (Mitamura et al. 2005; 2009). Therefore, the comparatively brief stocking period of this study would have no significant influence on rockfish homing ability. Gold code transmitters with pressure sensors (AQPX-1030P, AquaSound Inc., Kobe, Japan; 9.5 mm in diameter and 36 mm in length; sound pressure level: 155 dB re 1 µPa at 1 m; transmitting interval: ~2.5 s) were surgically inserted into the abdominal cavities of the fish under 0.1% 2-phenoxyethanol anaesthesia. During the operations, the rockfish were placed between layers of bubble wrap in fresh aerated seawater baths. After the surgery, the fish were kept in fresh seawater until the anaesthetic wore off.

The twelve tagged fish were categorised into two release types: 'group (G)' and 'single (S)'. G consisted of four individuals whereas there was only one in S (Table 4-1). Tagged fish were assigned to each release set (G1–2 or S1–4). They were placed in an inverted transparent container equipped with small holes and maintained on the sea bottom (~2.8 m depth) at a release point inside the array ~110 m away from the floating pier (Fig. 4-2). The release point was assumed to be outside the tagged fish home range because black rockfish generally inhabit rocky areas whereas the release point was situated on a flat sea bottom consisting of muddy sediment. After >30 min acclimation, the container was slowly opened and

the tagged fish were released either in groups (G1–2 in Table 4-1) or individually (S1–4 in Table 4-1) on 3 July 2017 and on 7 July 2017. Their 3D positions were monitored for ~3 days after release.

**Table 4-1.** Descriptive characteristics and summary of homing for tagged black rockfish *Sebastes cheni.*

| Fish ID[a] | TL[b] (cm) | BW[c] (g) | Fishing place[d] | Homing[e] | Duration (s) | Distance travelled (m) | Release day and time | Goal time |
|---|---|---|---|---|---|---|---|---|
| G1-1 | 22.0 | 181.5 | North, middle | Y | 7992.5 | 425.8 | | 20:37:22.5 |
| G1-2 | 23.0 | 190.5 | North, far | N | n/a | n/a | 2017/7/3 18:24:10 | n/a |
| G1-3 | 19.5 | 123.5 | North, middle | Y | 890.0 | 328.4 | | 18:39:00.0 |
| G1-4 | 19.5 | 119.5 | North, middle | N | n/a | n/a | | n/a |
| G2-1 | 21.7 | 165.5 | North, middle | Y | 2152.5 | 961.1 | | 19:05:53.5 |
| G2-2 | 20.5 | 156.5 | North, vicinity | N | n/a | n/a | 2017/7/6 18:30:00 | n/a |
| G2-3 | 19.3 | 109.5 | South, middle | Y | 2985.0 | 1090.8 | | 19:19:45.0 |
| G2-4 | 22.0 | 164.5 | South, middle | Y | 1867.5 | 441.7 | | 19:01:08.5 |
| S1 | 21.0 | 147.5 | North, far | N | n/a | n/a | 2017/7/3 18:35:20 | n/a |
| S2 | 21.0 | 148.5 | North, far | N | n/a | n/a | 2017/7/3 18:45:50 | n/a |
| S3 | 23.0 | 210.5 | North, middle | Y | 2595.0 | 337.0 | 2017/7/6 18:41:10 | 19:24:25.0 |
| S4 | 20.8 | 131.5 | North, middle | Y | 1852.5 | 585.6 | 2017/7/6 18:51:15 | 19:22:07.5 |

[a] Fish released in a group consisting of four individuals indicated as G1 or G2. Fish released alone was indicated as S1–4.

[b] Total length.

[c] Body weight.

[d] Relative direction and distance from the floating pier.

[e] Homing individuals indicated as 'Y'; non-homing individuals indicated as 'N.'

## 4.2.3 Data analysis

The horizontal and vertical components of the 3D positions of the rockfish were obtained separately. Horizontal positions were calculated by hyperbolic positioning method developed in the previous chapter. Vertical positions (swimming depth) were determined using a pressure sensor in the transmitters. Outliers were removed from the position data by 3D speed limitation (10 TL s$^{-1}$ in this study). If the speed differential between two consecutive data points was >10 TL s$^{-1}$, the latter point was removed. Position data were spatially interpolated every 2.5 s if there were zero or one missing values between each data step (temporal interval between two consecutive data points <~5 s). This 2.5-s interpolated position data was used in further analysis. Positioning was performed in Matlab R2017a (The Math Works, Natick, MA, USA).

Homing individuals were identified as those who returned to the fishing area around the floating pier where they were captured (area = 1,520.4 m$^2$; Fig 4-2). Once a tagged fish returned to the fishing area and remained there for 1 h, it was judged to be a homing individual. The home ranges of the tagged fish were estimated to establish whether their release points were outside and the fishing area was inside their home ranges. The 95% home ranges were calculated by kernel density estimation with adehabitatHR package for R v. 3.4.2 (R core team, 2017). Interpolated position data following sunrise on the day after the release (5:03 on 4 July 2017 or 5:05 on 8 July 2017) was used for estimation to exclude the influence of displacement. Inter-individual distances were calculated to determine whether the group type tagged fish travelled together. Distance travelled and travel time of a homing route were calculated for the homing individuals.

The movements of individuals in pairs were determined from instantaneous inter-individual distances and trajectories. To express collective fish movement, dynamic interaction (*di*; Long and Nelson, 2013) was calculated in the 2D plane. Dynamic interaction (*di*) measures

collectiveness between pairs of moving objects using Euclidian distances (step length) and azimuths (movement direction) between two consecutive positions. It was determined as follows:

$$di_t = f_t(v_t^a, v_t^b) = di_\theta \times di_d$$

$$di_\theta = g_t(\theta_t^a, \theta_t^b) = \begin{cases} 0 & \text{one of } \theta_t^a \text{ or } \theta_t^b \text{ undefined} \\ 1 & \text{both } \theta_t^a \text{ and } \theta_t^b \text{ undefined} \\ \cos(\theta_t^a - \theta_t^b) & \text{otherwise} \end{cases}$$

$$di_d = h_t(d_t^a, d_t^b) = \begin{cases} 1, & d_t^a + d_t^b = 0 \\ 1 - \left(\dfrac{|d_t^a - d_t^b|}{d_t^a + d_t^b}\right)^\alpha, & d_t^a + d_t^b > 0 \end{cases}$$

where $v_t$ is the vector at time step $t$, $\theta_t$ is the angle of movement at time step $t$, $d_t$ is the Euclidian distance at time step $t$, $|\cdot|$ is the absolute value operator, and $\alpha$ is the scaling parameter (set to 1 in this study). Using this definition, both $di_\theta$ and $di_d$ have a range of [-1, 1]. Therefore, $di_t$ has the same range. Positive values of $di_t$ correspond to cohesive or positively interactive movements while negative values are interpreted as repulsive or opposing movements. Values near zero mean no interaction. For the segments of the homing routes where individuals moved together, the individual at the frontal position relative to the movement direction and step length of the pair was determined. In addition, the positional relationship index (*PR*) of the pair with respect to the ratio of movement direction to inter-individual distance was calculated as follows:

$$PR = \frac{d_{dir}^2}{d_{int}^2}, \quad d_{int} > 0$$

where $d_{dir}$ is distance in movement direction and $d_{int}$ is the inter-individual distance (between the pair of fish). *PR* has a range of [0, 1]. Values near one mean that pairs are parallel to the movement direction whereas values near zero are pairs are perpendicular to the movement direction.

To compare homing success rate, distance travelled, and travel duration between release types and among TL and BW, a hierarchical Bayesian model was constructed. For the homing success rate,

$$p(Z_i \mid \theta_i) \sim Bernoulli(\theta_i)$$
$$logit(\theta_i) = \alpha + \beta_1 * type_i + \beta_2 * TL_i + idv_i$$
$$p(idv_i) \sim Normal(0, \sigma^2)$$

where $Z_i$ is a binary indicator of homing success following the Bernoulli distribution with mean $\theta_i$ expressed as a linear predictor with a logit-link function. In the linear predictor, $type_i$ is a binary indicator of two release types (group or single), and $TL_i$ is mean-centred total length. The individual difference $idv_i$ is expressed *a priori* following a normal distribution with mean 0 and variance $\sigma^2$. Only TL was included in the linear predictor because BW was highly correlated with TL (r = 0.95). For the distance travelled and the travel duration, the same Bayesian model was applied:

$$p(D_i \mid \mu_i, \tau) \sim Gamma(\mu_i * \tau, \tau)$$
$$log(\mu_i) = \alpha + \beta_1 * type_i + \beta_2 * BL_i + idv_i$$
$$p(idv_i) \sim Normal(0, \sigma^2)$$

where $D_i$ is distance travelled or travel duration following a Gamma distribution with mean $\mu_i$ and scale parameter $\tau$. The mean $\mu_i$ was expressed as a linear predictor with a log-link function. The parameters $type_i$, $TL_i$, and $idv_i$ were the same as those used for the homing success model. The probability distributions of $\alpha$, $\beta_1$, and $\beta_2$ were estimated from 100,000 MCMC samplings with three chains after 10,000 burn-in iterations. Bayesian analysis was performed using JAGS (Hornic, 2003) in the rjags package (Plummer 2016) of R v. 3.4.2 (R core team, 2017).

The movement directions of every fish relative to the current just after release (inside the bay) and on the way to the fishing area (outside the bay) were investigated. Current data were unified for 18:00–19:00 (just

after release and on the way to the fishing area), 19:00–20:00, and 20:00–21:00 (on the way to the fishing area). The null hypothesis that the current direction is uniformly distributed was evaluated by the Rayleigh test. The movement directions for the trajectories of every fish were visually determined.

The associations between swimming depth and bottom depth on the routes of the homing individuals were investigated. A linear model (LM) was constructed in R v. 3.4.2 (R core team, 2017). The swimming depth at a localised point was used as the response variable and the bottom depth at a localised point was used as the explanatory variable. The intercept was set to zero. The bottom depth was derived from the depth contour data.

## 4.3 Results

Seven of twelve tagged black rockfish successfully returned to the fishing area during the night of the release day (Fig. 4-3 and Table 4-1). For the group release types, two (G1-1 and G1-3) and three (G2-1, G2-3, and G2-4) of four individuals returned to the fishing area (Fig. 4-3 and Table 4-1). For the single release type, two of four individuals (S3 and S4) returned to the fishing area (Fig. 4-3 and Table 4-1). Differences in release type and TL did not significantly affect homing success rate (Table 4-3). The remaining five of the twelve tagged fishes did not return to the fishing area. Two of them (G1-4 and G2-2) left the receiver array and did not return to it within the ~3-d monitoring period after release (Fig. 4-3). Another three individuals (G1-2, S1, and S2) stayed outside the fishing area (Fig. 4-3).

For the seven homing individuals, the effects of release type and TL on travel duration and distance travelled were investigated. The travel duration was 2,905 ± 2,337.9 s (mean ± s.d.; n = 7 individuals; range: 890–7,992.5; Table 4-1) and the distance travelled was 595.8 ± 308.1 m (n = 7; range: 328.4–1090.8; Table 4-1). Differences in release type and TL had no significant effects on travel duration or distance travelled (Table 4-3).
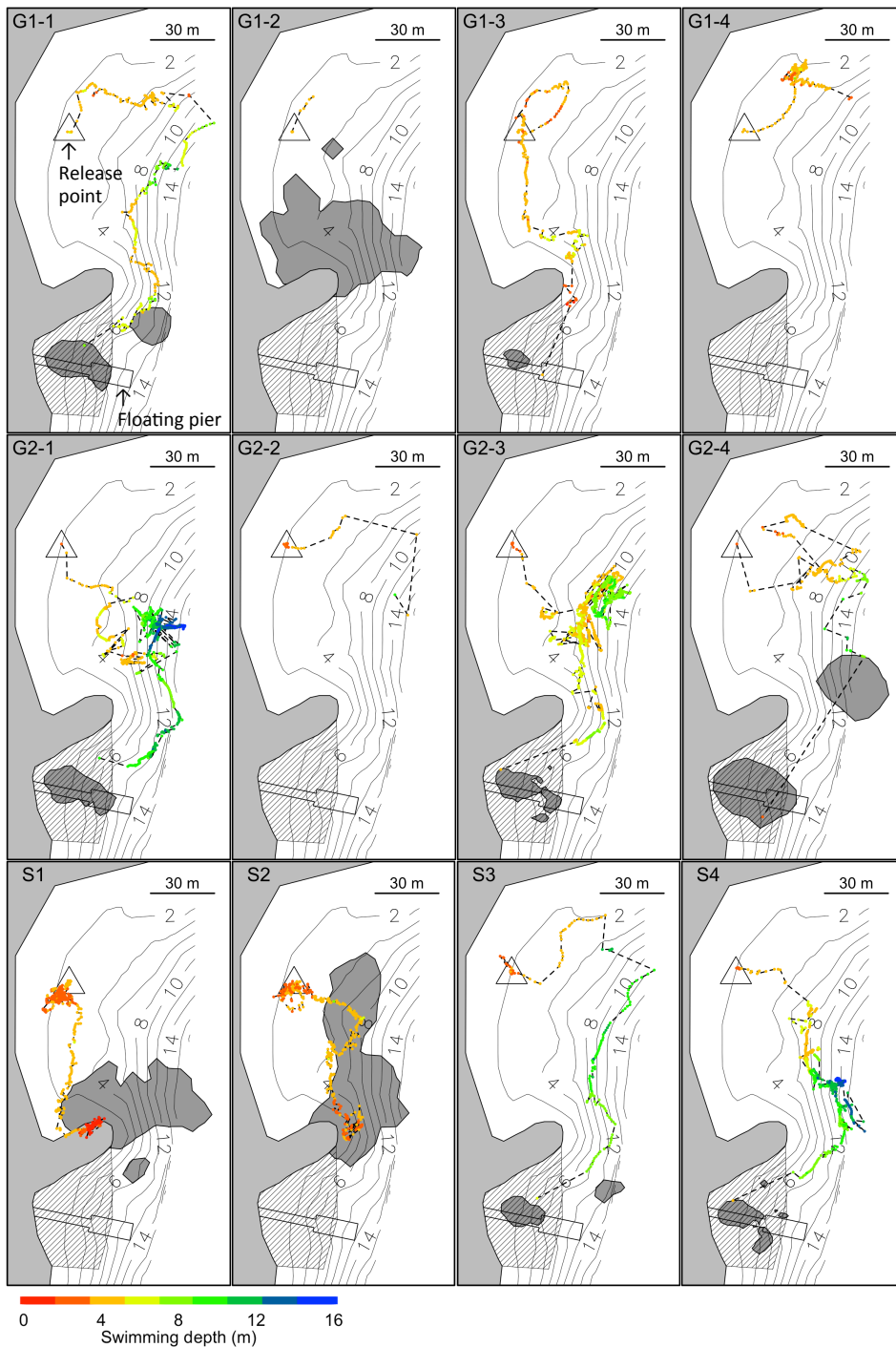
**Fig. 4-3.** Travelling routes and/or home ranges of twelve tagged black rockfish. G1-1–4 or G2-1–4 were released simultaneously whereas S1–4 were released individually. The cross-hatched area around the floating pier is the home area where the tagged rockfishes were captured. Dotted lines with open circles indicate homing routes (G1-1, G1-3, G2-1, G2-3, G2-4, S3, and S4), localised positions (G1-4, and G2-2), or localised positions within 1 h after release (G1-2, S1, and S2). Colour variations indicate swimming depth. Area of 95% home ranges is indicated in dark grey.

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The home ranges were estimated for all but two tagged black rockfish. G1-4 and G2-2 left the receiver array; so, their home range areas could not be calculated. The area of the 95% home range was 981.1 ± 876.7 m$^2$ (n = 10 individuals; range: 81.2–2,267.2; Table 4-2). The release point was outside their home ranges. Therefore, the tagged fishes were released and travelled from an unfamiliar area after their displacement (Fig. 4-3). The home ranges of the seven homing individuals were included as part of the fishing area. In contrast, those of the three non-homing individuals were outside the fishing area (Fig. 4-3).

According to their inter-individual distances and movement trajectories, the tagged black rockfish released in groups did not travel with other individuals for most of their journey. The tagged fish were separated from each other by tens of metres for some time after their release (Fig. 4-4). Additionally, the individually released tagged fish did not travel with the other individuals released the same day (Fig. 4-5 and 4-6). However, G1-3 and G1-4 travelled together for ~100 s after release (Fig. 4-7). G1-4 started to move ~30 s after release and G1-3 followed and eventually caught up with G1-4. They then swam synchronously along the sea bottom for ~50 s. The *di* was very high (near unity) (Fig. 4-7F). During the synchronous movement, the leader and follower traded places several times (Figs. 4-7F–G). *PR* were near zero, so they moved side by side (Fig. 4-7G). After a separation, G1-3 changed direction and returned home (Fig. 4-3) whereas G1-4 settled in one place for a while until it was separated from G1-3 and left the receiver array (Fig. 4-3).

The direction of rockfish movement relative to the current was investigated. Around the release time (18:00–19:00) on 3 July 2017, the current was directed towards the northeast (Fig. 4-8 and Table 4-4). G1-1 and G1-2 moved towards the north while G1-3 and G1-4 headed northeast. On the other hand, S1 and S2 wandered about the release point awhile then moved south and east, respectively (Fig. 4-3). Around the release time (18:00–19:00) on 6 July 2017, the current was not concentrated in any direction (Fig. 4-8 and Table 4-4). G2-1, G2-3, and G2-4 moved south, S4

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went east, G2-2 headed northeast, and S3 returned to the east after having going back and forth from the northwest (Fig. 4-3). For the homing journey of the seven individuals, the currents outside the bay were either directed towards the west (3 July 2017; and 19:00–20:00 on 6 July 2017; Fig. 4-8 and Table 4-4) or not directed at all (18:00–19:00 in 6 July; Fig. 4-8 and Table 4-4). Their final direction towards the fishing area was along the bathymetry line.

Homing individuals swam mostly along the sea bottom on the way to their fishing area. The swimming depth was significantly correlated with the bottom depth (Fig. 4-9). The slope of the regression line was 0.7272 ± 0.136 (range: 0.5297–0.8733), which means that the homing individuals tended to travel along the sea bottom.

**Table 4-2.** Estimated 95% home range area ($m^2$) of twelve tagged black rockfish after sunrise the day after release.

| Fish ID[a] | Number of positions used in estimation | 95% home range ($m^2$) |
|---|---|---|
| G1-1 | 70374 | 649.1 |
| G1-2 | 57803 | 2228.1 |
| G1-3 | 72977 | 81.2 |
| G1-4 | 0 | n/a[b] |
| G2-1 | 28391 | 405.6 |
| G2-2 | 0 | n/a[b] |
| G2-3 | 42469 | 301.5 |
| G2-4 | 63046 | 1520.5 |
| S1 | 68025 | 1816.1 |
| S2 | 100812 | 2267.2 |
| S3 | 37638 | 257.2 |
| S4 | 31865 | 284.2 |
| Mean ± s.d.[c] | 57340 ± 22478.4 | 981.1 ± 876.7 |

[a] Fish released in a group consisting of four individuals is indicated as G1 or G2. Fish released alone indicated as S1–4.

[b] Home range was not estimated because the fish left the receiver array.

[c] Calculated for all except G1-4 and G2-2.

**Table 4-3.** Estimates of the intercept and coefficients in the regression for homing success rate, travel duration, and distance travelled by MCMC sampling.

| Response variable | Term | Mean | 2.5% | 97.5% | Gelman-Rubin statistics |
|---|---|---|---|---|---|
| Homing success rate | intercept | 19.17 | -147.60 | 178.00 | 1.00 |
| | type | -7.01 | -180.80 | 172.30 | 1.00 |
| | TL | 9.19 | -151.20 | 165.50 | 1.00 |
| Travel duration | intercept | 7.91 | 6.96 | 9.08 | 1.14 |
| | type | -0.30 | -1.94 | 1.19 | 1.03 |
| | TL | 0.25 | -0.35 | 0.88 | 1.04 |
| Distance travelled | intercept | 6.29 | 5.47 | 7.03 | 1.00 |
| | type | -0.07 | -1.55 | 1.86 | 1.03 |
| | TL | -0.12 | -0.72 | 0.47 | 1.00 |
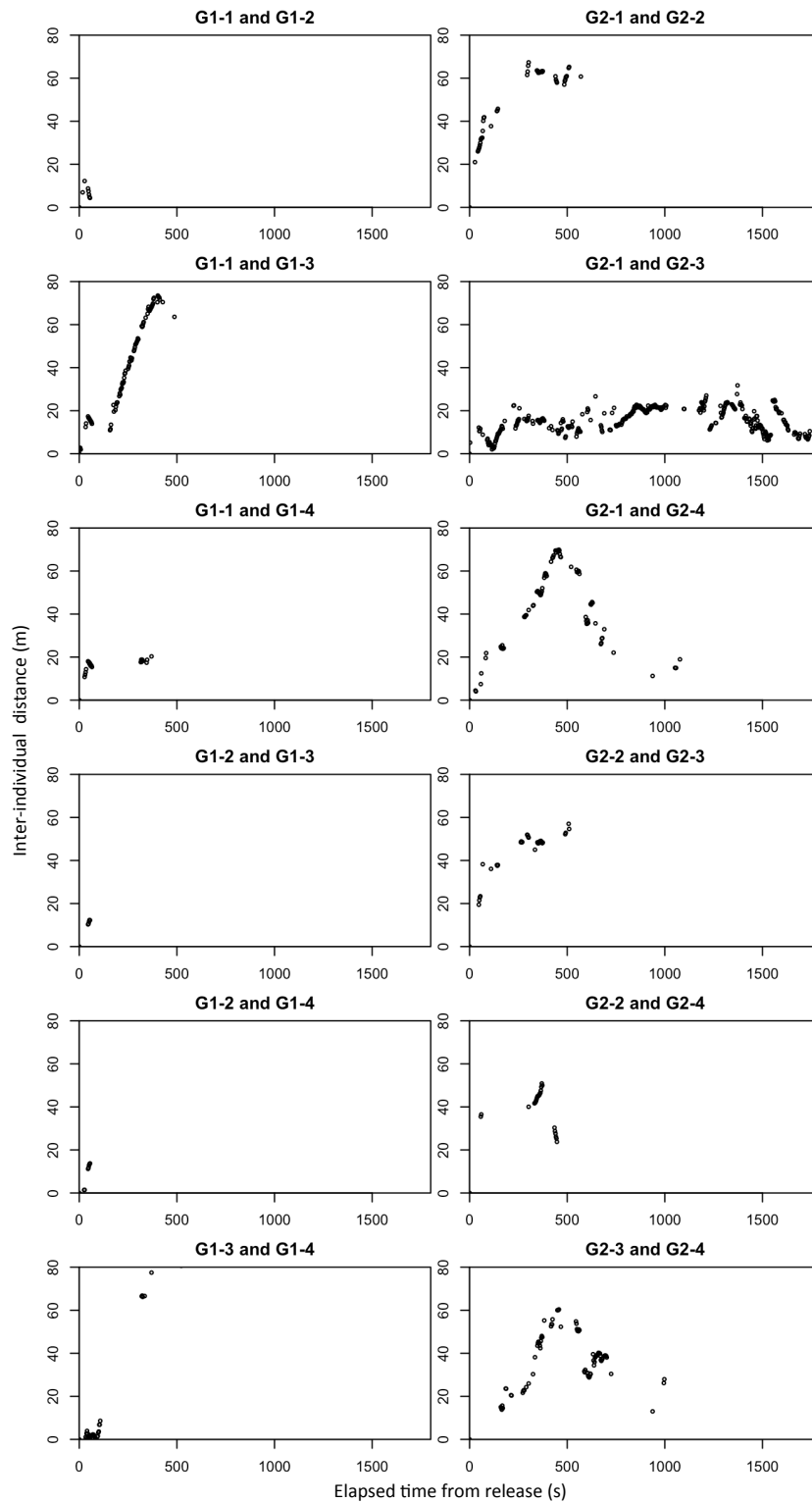
**Fig. 4-4.** Inter-individual distances of G1 and G2 black rockfish 1,800 s after release. The graph titled 'G1-1 and G1-2' shows time series distance between fish G1-1 and fish G1-2.
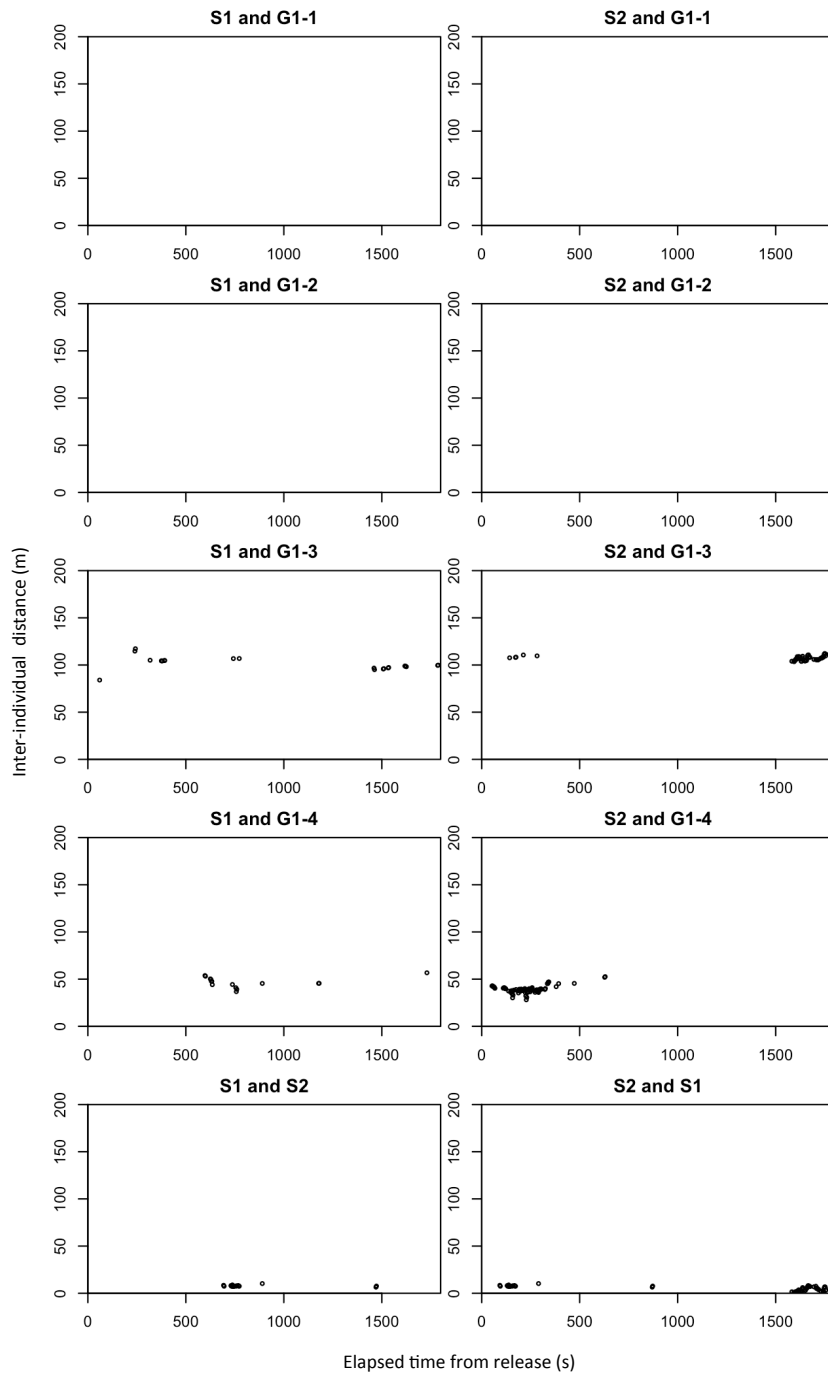
**Fig. 4-5.** Inter-individual distances of S1, S2 and G1 black rockfish 1,800 s after release. The graph titled 'S1 and G1-1' shows time series distance between fish S1 and fish G1-1.
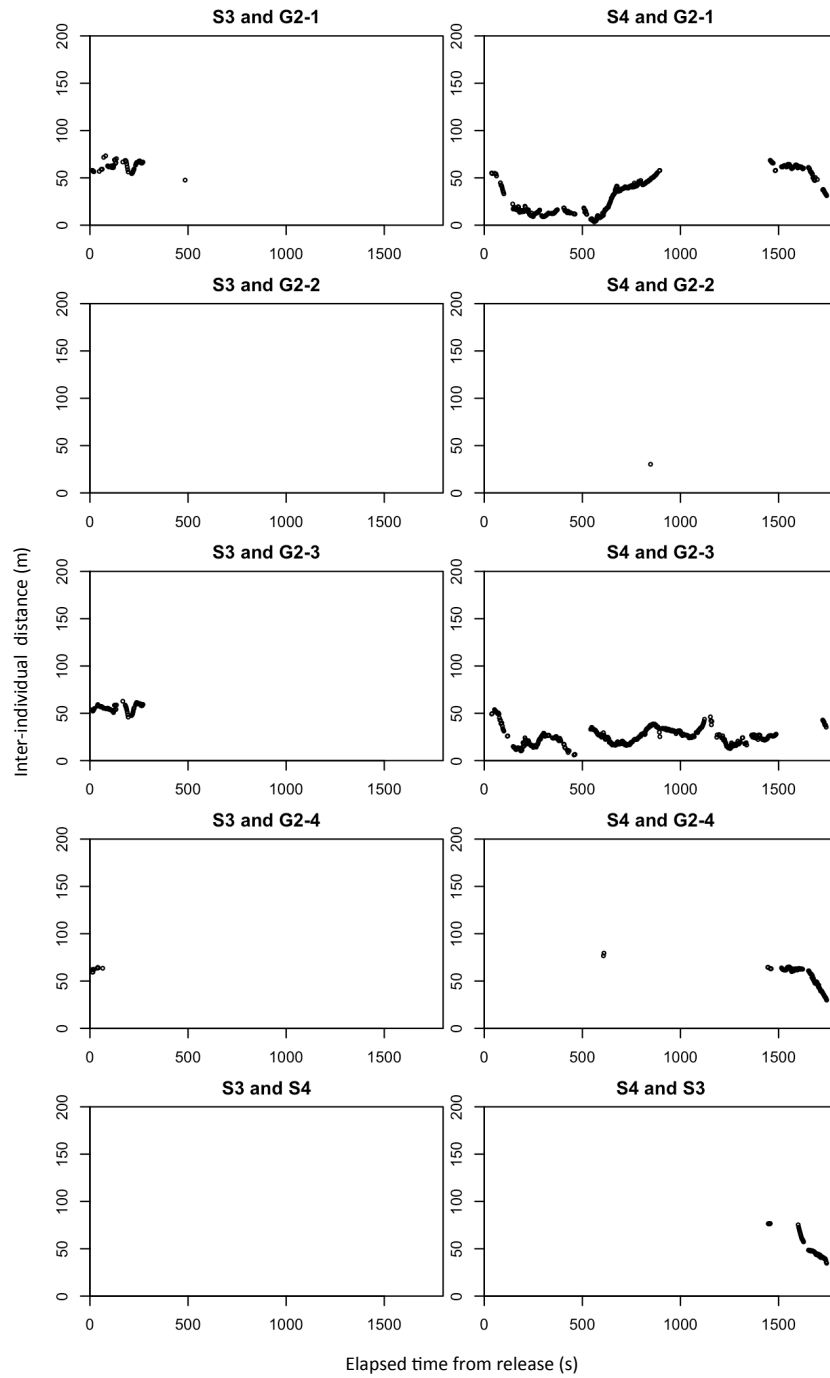
**Fig. 4-6.** Inter-individual distances of S3, S4 and G2 black rockfish 1,800 s after release. The graph titled 'S2 and G2-1' shows time series distance between fish S2 and fish G2-1.
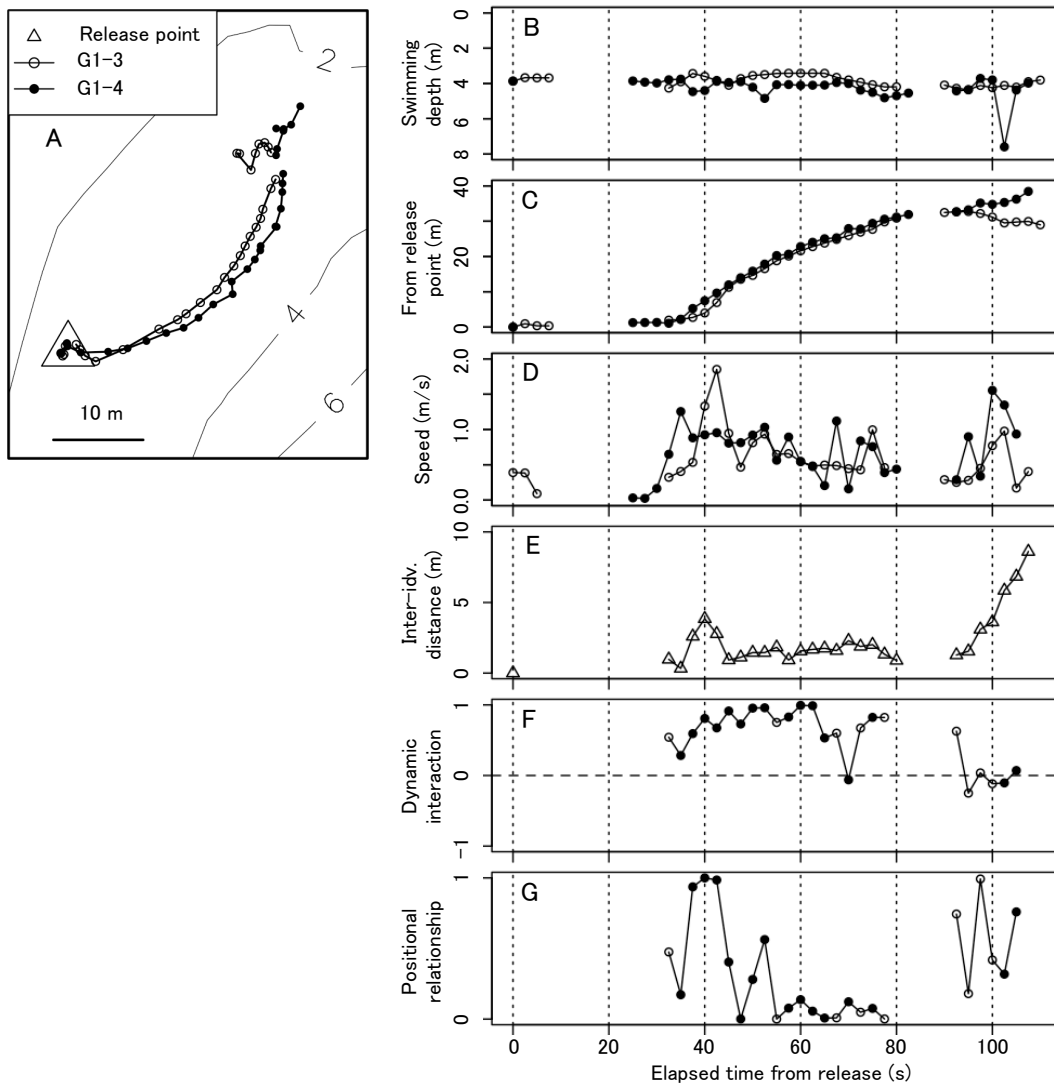
**Fig. 4-7.** Schooling behaviour of G1-3 and G1-4 for 110 s after release. White and black circles indicate G1-3 and G1-4, respectively. (A) 2D positions. Thin lines of 2 m, 4 m, and 6 m indicate depth contours. (B) Swimming depth. (C) Distance from release point. (D) 3D movement speed. (E) 2D inter-individual distance. (F) Dynamic interaction between two fish. Positive values correspond to cohesive or positively interactive movements. Negative values indicate repulsion or opposing movements. Values near zero mean no interaction. (G) Positional relationship between two fish. Values near one mean pairs travelling parallel to the movement direction. Values near zero mean pairs travelling perpendicular to the movement direction. In (F) and (G), white or black circle indicates that G1-3 or G1-4 is following the other in the movement direction.

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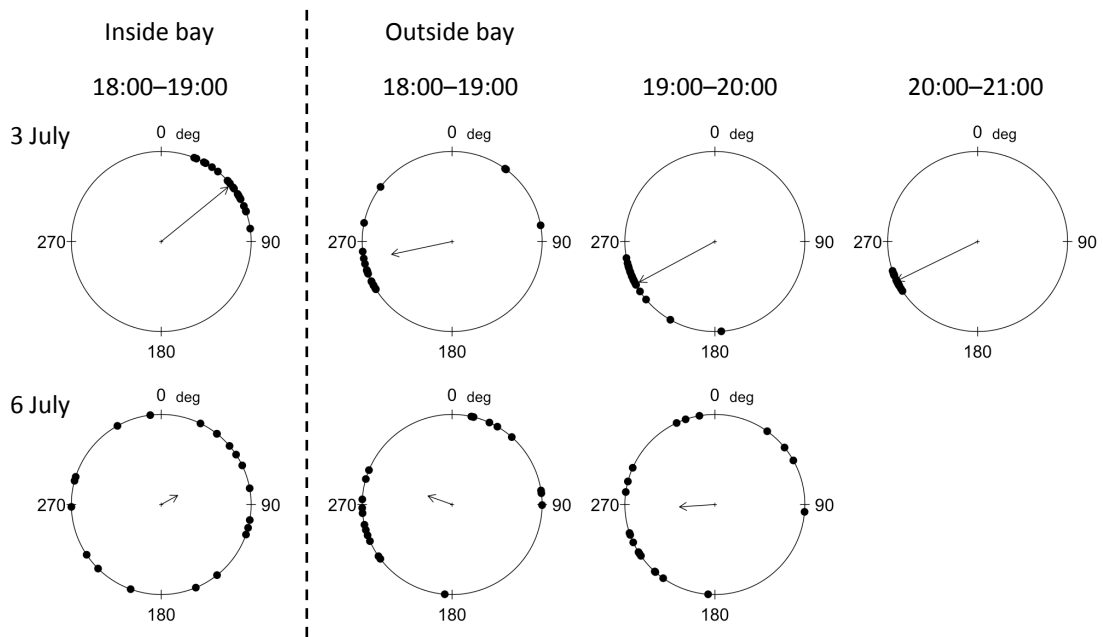
**Fig. 4-8.** Circular distribution of current direction at two points (inside/outside bay) at 18:00–19:00, 19:00–20:00, and 20:00–21:00 on two release days (3 July 2017 and 6 July 2017). Black circles indicate current direction at 3-min intervals. Arrows indicate mean direction.

**Table 4-4.** Current direction at two points (inside/outside bay) at 18:00–19:00, 19:00–20:00, and 20:00–21:00 on the release days 3 July 2017 and 6 July 2017.

| Day | Place | Time | Length (r) | Mean angle (deg) | Test statistic | p-value |
|---|---|---|---|---|---|---|
| 3 July | Inside | 18:00–19:00 | 0.96 | 51 | 0.96 | <0.001 |
| | Outside | 18:00–19:00 | 0.69 | 258 | 0.69 | <0.001 |
| | | 19:00–20:00 | 0.95 | 242 | 0.95 | <0.001 |
| | | 20:00–21:00 | 1 | 244 | 1 | <0.001 |
| 6 July | Inside | 18:00–19:00 | 0.21 | 61 | 0.21 | >0.05 |
| | Outside | 18:00–19:00 | 0.28 | 290 | 0.28 | >0.05 |
| | | 19:00–20:00 | 0.39 | 266 | 0.39 | <0.05 |

0 degree indicates northerly direction.

Length indicates the length of the mean vector ($0 \leq r \leq 1$).

The Rayleigh test was used.
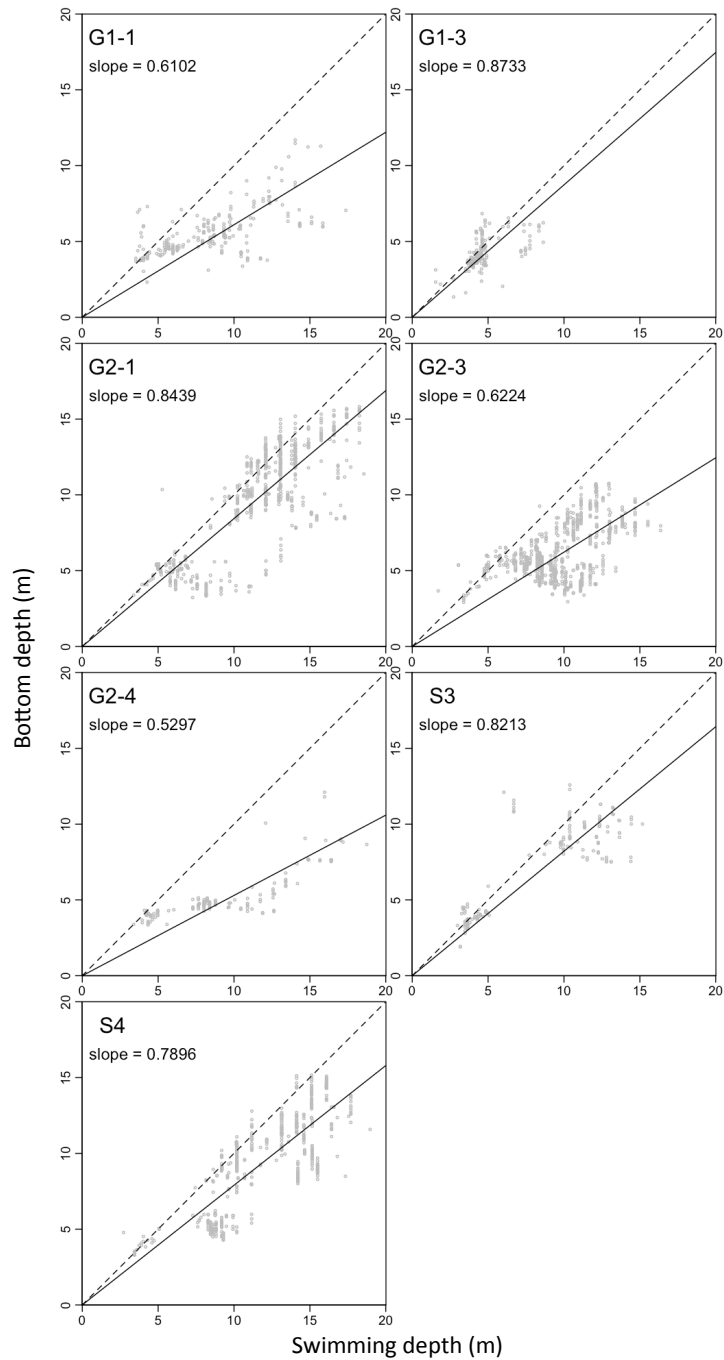
**Fig. 4-9.** Swimming depth vs. bottom depth in homing travel of seven black rockfish. Fish ID is shown at the top left of each figure: G1-1 (n = 256), G1-3 (n = 199), G2-1 (n = 660), G2-3 (n = 853), G2-4 (n = 145), S3 (n = 166), and S4 (n = 569). Diagonal dotted lines represent a 1:1 ratio of change. Regression line slopes are shown. Black lines represent regression lines.

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## 4.4 Discussion

Except for one case, the tagged black rockfish released in groups moved without other individuals. This behaviour contradicted my hypotheses. The exception was that G1-3 and G1-4 swam with each other for ~100 s after release (Fig. 4-7). Therefore, the first hypothesis (cohesive movement) was not rejected but was validated in a limited manner. G1-3 and G1-4 moved almost side by side and took turns leading and following (Fig. 4-7). As is the case for other self-organized animals like penguins (Nesterova et al., 2014) and pigeons (Biro et al., 2006), black rockfish have no designated leader when they move collectively. G1-3 and G1-4 were smaller than G1-1 and G1-2. The former were 2–3 cm smaller in TL and ~60 g lighter in BW than the latter (Table 4-2). In a preliminary experiment, black rockfish initially formed a group of 2–5 individuals when 16 individuals were released simultaneously (Takagi, personal observation; Fig. 4-1). They were relatively smaller (~15–18 cm in TL) than the specimens used in the actual experiment of this study. Specimens in the preliminary experiment and those in present study were >150 mm in total length and were considered adults (Mio, 1960). Juvenile black rockfish form large schools whereas the adults tend to be solitary and usually avoid schooling or shoaling (Harada, 1962). Unlike anchovies or mackerel, then, adult black rockfish do not have a strong tendency to form groups. However, smaller adult black rockfish may be more inclined to group than larger ones. Nevertheless, they would not exclude other individuals because black rockfish are not territorial except for the males in spawning season (October to January; Shinomiya and Ezaki, 1991) which was not part of my experimental period. There is no strong evidence that black rockfish never travel in schools or shoals. In the present study, only four individuals were simultaneously released. This number may have been too small to motivate other individuals to form a group.

My second hypothesis (effective homing in schooling fish) was not

supported in this study. There were no significant differences in homing rate, distance travelled, or homing duration between the group- and single-release types (Table 4-3). Social cues were less effective than olfactory or visual cues. There are several external factors driving animal movement (Nathan et al., 2008). In this experiment, social cues (movement direction of other conspecifics) were less effective at inducing homing than other cues. Black rockfish generally have small home ranges in rocky areas (Harada, 1962; Mitamura et al. 2009). Estimated home ranges were defined nearly by the rocky areas around the floating pier. They did not include the release site or the trajectory of the rockfish (Fig. 4-2). The home range was estimated using only 3 d of positional data. Therefore, the home range may have been underestimated. Rockfish selected one to three relatively narrow core areas within the wider home ranges (Jorgensen et al., 2006). Tagged rockfish may have home ranges wider than those estimated, and the homing path was included in their home range area. Their homing paths were similar along the bathymetry line. Therefore, they may use visual cues and landmarks for their homing. Mitamura et al. suggested that black rockfish relied on vision in familiar areas during their homing (Mitamura et al., 2009). Tagged rockfish may memorise daily landmarks in their home ranges and depend on those rather than social cues to return to their place of origin. In contrast, they may rely on social cues when they are displaced in apparently unfamiliar areas. In fact, it is possible that black rockfish never use social cues from other conspecifics. G1-3 and G1-4 started to move unexpectedly in the same direction and travelled along the same path. However, this experiment did not prove that their movements did not mutually affect each other.

Seven of the twelve tagged black rockfish exhibited homing behaviour from an unfamiliar area to the fishing area. When the current direction was the most concentrated at the release date (3 July 2017), four G1 individuals moved along the current direction just after release. In contrast, S1 and S2 wandered around the release point for a while then moved in a direction different from that of the current (Fig. 4-3). On the

contrary, when the current direction was not concentrated at the release date (6 July 2017), G2-1–4, S3, and S4 moved in all directions (Fig. 4-3). Black rockfish may use olfactory cues for homing from an unfamiliar area (Mitamura et al., 2005). They move upstream, downstream, and back and forth to approximate homing direction by odour (Mitamura et al., 2011). The movements of four individuals in the G1 release type were consistent with earlier predictions. Odour dispersal may be complex on a local scale because of the shifting local current. Therefore, individuals may find olfactory cues in the changing local current and change direction with it. Homing individuals travel in similar trajectories towards the end of their journeys along the bathymetry line and the sea bottom independently of current direction. Nevertheless, their paths are apparently influenced by bottom depth (Figs. 4-3 and 4-9). Home range may be underestimated, and rockfish may explore areas out of their home range to search for new habitats (Matthews, 1990b). Homing individuals may memorise landmarks in their daily life or during migrations. It is suggested that black rockfish may use visual cues for homing in familiar areas (Mitamura et al., 2009). The probability of finding landmarks would be high along the sea bottom. Homing rockfish evidently use landmarks to navigate back to the fishing area.

Five of the twelve rockfishes did not return to the fishing area during the 3-d monitoring period. G1-4 and G2-2 left the receiver array and were never detected by the receivers after departure. Rockfish were captured ~10–20 m north of the floating pier (G1-4; Table 4-1) and next to the floating pier (G2-2; Table 4-1). Other individuals captured at the same place as G1-1, G1-3, G2-1, S3, and S4 returned to the fishing area and stayed there (Fig. 4-3). In previous studies, several black rockfish did not home (Mitamura et al., 2002). Others homed only several days after release (Mitamura et al., 2005). Black rockfish inhabited certain rocky areas north of the receiver array. G1-4 and G2-2 might have settled in another suitable place outside the receiver array or returned to the fishing area after the monitoring period. G1-2, S1, and S2 settled in an area on a groyne head

north of the fishing area with a wider home range than that of the homing individuals (Fig. 4-3 and Table 4-2). These three rockfish were captured relatively far from the floating pier near the groyne head (Table 4-1). Their home range area was relatively wide. Therefore, they may have been captured on the edge of their home range overlapping with the fishing area.

This study tested two hypotheses: (1) black rockfish form groups in their homeward journeys, and (2) homing in a group improves migratory efficiency in the natural environment. Only one pair of rockfish were found to travel together just after release. Therefore, improvement of homing efficiency by group travel was not proven in the present study. However, this study may serve as a catalyst for further investigation into the collective navigation of fish in the natural environment.

## 4.5 Conclusions

In this chapter, my hypotheses of collective movement in homing black rockfish were tested using fine-scale acoustic positioning method developed in the previous chapter. Two fish moved together despite short period of ~100 s, and thereby my hypothesis for collective movement was partly proved. However, my hypothesis for improvement of migrating efficiency was not supported. In the next chapter, as the consequence of this thesis, I discuss contribution of this challenge and newly developed positioning method.

# Chapter 5

# **General discussion**

## **5.1 Contribution to future study**

Through this research, I attempted to observe collective movement behaviour in a coastal fish that has site-fidelity and homing ability, and tested hypotheses concerning its potential collective navigation, through the development and application of a fine-scale positioning method using biotelemetry techniques. As a result, I observed that black rockfish did not show schooling behaviour leading to enhanced navigational ability by collective movement. However, results did demonstrate that the method I developed was able to observe whether or not the rockfish moved in a school; thus, the method can potentially be used to observe schooling, i.e., group behaviour, in fish. This study is thus a pilot study that demonstrated the observation of collective movement behaviour by fish by collecting precise and accurate positional data in a natural environment.

The positioning method developed herein will be a powerful tool, which should be able to provide empirical data on the group behaviour of aquatic animals in various fields. Almost all studies on group behaviour in fish have relied on video observations or echo sounders, which often have restricted ranges in space and time (e.g., Marras et al. 2015, Bui et al. 2013). Receivers being deployed in closed environments, or widely and densely in open water, will be able to provide more constant information on the

positions of individuals than ever before using the method developed in this thesis. Few studies have been conducted that used data loggers to reveal the mysteries of collective movement in aquatic animals. For example, Noda et al. (2016) elucidated that individual differences in gliding behaviour by schooling Bluefin tuna (*Thunnus orientalis*) were derived from differences in body size through observations made in a fish pen. Adequate and novel results can thus be obtained using data-loggers. However, if receivers were deployed in a fish pen, then positional relationships among individuals could be obtained using the method developed herein, which would allow for more detailed information to be collected. Linking biotelemetry and bio-logging techniques should lead to new insights into fish behaviour from the merging of such information, i.e., what the fish do and where they do it.

The empirical data that will be obtained using this newly developed positioning method in the future will enhance the coupling of the two disciplines of movement ecology and the study of collective movement. Delgado et al. (2014) suggested that the main limitation to inferring the influence of conspecifics on movement behaviour was the need for high-resolution movement data acquired simultaneously from a sufficiently large number of individuals. GPS technology has advanced to the point that we can adequately observe multiple terrestrial animals simultaneously. However, the simultaneous acquisition of movement data for multiple aquatic animals has remained difficult. The positioning method I developed will help researchers to obtain the needed high-resolution movement data for multiple aquatic animals, although the scope of the data collected will be restricted by the receiver array. In most cases, this issue should be resolvable by proper experimental design. Additionally, the positioning method developed will enable researchers to observe inter- and intra-specific interactions, including not only schooling behaviour, but also prey-predator interactions or mating behaviour, based on fine-scale positional data.

## 5.2 General conclusions

In this thesis, I developed a fine-scale positioning method utilizing novel technology and biotelemetry techniques, which can simultaneously pinpoint multiple aquatic animals with a fine-scale spatial (<10 cm) and temporal (<10 s) resolution. I then applied the positioning method I developed to testing hypotheses of homing behaviour by black rockfish in the field based on these animals' potential collective navigation. Black rockfish, however, did not exhibit collective movement, at least not in this experiment. In this way, although the hypotheses were only partially supported, I could demonstrate that the positioning method I developed could localize fish precisely enough to potentially observe collective movement behaviour. This study offered a possible method that can be used to observe the collective movement of fish in the field. The positioning method developed in this thesis has great potential to contribute empirical data on the group behaviour of fish, including not only schooling behaviour by conspecifics but also inter- and intra-specific interactions. These data will help advance efforts to link movement ecology and the study of group behaviour.

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[1]. Takagi, J., Ichikawa, K., Arai, N., Miyamoto, Y., Uchida, K., Fujioka, K., Fukuda, H., Shoji, J., and Mitamura, H. (2016) Simultaneous identification of multiple signals from phase modulation-coded transmitters for acoustic biotelemetry of fish school. *Journal of Advanced Marine Science and Technology Society, 22*(2): 5–9.

[2]. Takagi, J., Ichikawa, K., Arai, N., Miyamoto, Y., Uchida, K., Fujioka, K., Fukuda, H., and Mitamura, H. (2017). Evaluation of multipath effects on depth measurements provided by acoustic transmitters in shallow water. *PROCEEDINGS of the Design Symposium on Conservation of Ecosystem (The 16th SEASTAR2000 workshop), 5*: 15–19.

[3]. Takagi, J., Ichikawa, K., Arai, N., Miyamoto, Y., Uchida K., Shoji, J., and Mitamura, H. (2018). Simultaneous observation of intermittent locomotion of multiple fish by fine-scale spatiotemporal three-dimensional positioning. *PLoS ONE*, 13(7): e0201029.

[4]. Takagi, J., Ichikawa, K., Arai, N., Miyamoto, Y., Uchida K., Shoji, J., and Mitamura, H. (In preparation). Do sympatric conspecific fish use social cues in homing? An approach by fine-scale acoustic positioning