Journal of the Marine Biological Association of the United Kingdom

cambridge.org/mbi

Quantitative relationship of Sr:Ca of statoliths of the Japanese flying squid (*Todarodes pacificus*) with empirical water temperatures

Tadanori Yamaguchi[1], Hajime Matsui[2], Hisae Miyahara[2], Hiroshi Kubota[3] and Naoki Hirose[1,4]

[1]Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, Kasuga, Japan; [2]Yokohama Field Station, Pelagic Fish Resources Division, Fisheries Stock Assessment Center, Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Yokohama, Kanagawa, Japan; [3]Yokohama Field Station, Highly Migratory Resources Division, Fisheries Stock Assessment Center, Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Yokohama, Kanagawa, Japan and [4]Research Institute for Applied Mechanics (RIAM), Kyushu University, Kasuga, Japan

Research Article

Cite this article: Yamaguchi T, Matsui H, Miyahara H, Kubota H, Hirose N (2025). Quantitative relationship of Sr:Ca of statoliths of the Japanese flying squid (*Todarodes pacificus*) with empirical water temperatures. *Journal of the Marine Biological Association of the United Kingdom* **105**, e14, 1–11. https://doi.org/10.1017/S0025315425000098

Received: 21 May 2024
Revised: 4 November 2024
Accepted: 18 January 2025

Keywords:
Lagrangian tracer experiment; migration; statolith; *Todarodes pacificus*; empirical water temperature

Corresponding author:
Tadanori Yamaguchi;
Email: tadanoriyama@riam.kyushu-u.ac.jp

Abstract

The Japanese flying squid, *Todarodes pacificus*, is distributed mainly in the northwest Pacific and the Japan Sea. The present study was conducted for a better understanding of the mechanisms behind its migration routes. The ratios of strontium to calcium (Sr:Ca) in the statoliths can be associated with the water temperatures the squid experienced in the sea. Using specimens collected in the northern Japan Sea in summer and Lagrangian backward tracer experiments, a strong negative correlation was obtained between the Sr:Ca in the statoliths and the empirical water temperatures estimated through a regional ocean model. These backward tracer experiments were continuously conducted at depths of 6, 15, and 30 m. The greatest determination coefficient of the regression expression appeared for a near-shore tracer group of the experiment at a depth of 15 m. In addition, the regression expression provided reasonable lifetime empirical water temperature variations of the squids collected in the sea areas east of Tsushima Island and west of the Goto Islands in winter. The combination of Ca:Sr analytical chemistry and tracer experiments with the ocean dynamic model used in this study improved our understanding of the migration path of *T. pacificus*.

Introduction

Todarodes pacificus (the Japanese flying squid) is distributed in the middle latitudes of the Northwest Pacific, including the Japanese Archipelago and the Korean Peninsula. It migrates between its spawning grounds (in the southwestern Japan Sea, Tsushima Strait, and the northeastern East China Sea) and feeding grounds (the waters around Hokkaido Island including the Sea of Okhotsk) during its 1-year life cycle (Okutani, 1983; Arkhipkin *et al.*, 2015; Gleadall *et al.*, 2024). The stock of this species around Japan includes three cohorts based on the spawning period (summer, autumn, and winter), with those caught in the Japan Sea mainly belonging to the autumn cohort (Rosa *et al.*, 2011). The eggs of this cohort are spawned in the southern Japan Sea and the Tsushima Strait between October and December (Sakurai *et al.*, 2013). The hatchlings are transported northeastwards along with the Tsushima Warm Current to the waters off Hokkaido Island and grow in subarctic waters during the summer before migrating back to the Tsushima Strait for spawning.

The annual catches of the autumn cohort of *T. pacificus* increased in the 1990s after a decrease between the mid-1970s and mid-1980s. The long period of poor catches of *T. pacificus* was perhaps caused not only by excessive fishing effort in the Japan Sea but also by changing environmental conditions (Murata, 1989; Sakurai *et al.*, 2000). The recovery of this cohort from the period of poor stock was probably triggered by an expansion of the spawning grounds with the 1989 regime shift (Kidokoro *et al.*, 2010); resulting in the catches off Japan increasing to 178,143 tonnes (t) in 1996 (Goto, 2002). However, the annual catches of Japan have gradually decreased again since around 2000, dropping to 13,416 t in 2019 (Miyahara *et al.*, 2023). The drastic drop in landings from around Hokkaido could be accounted for not only by the decline of the autumn cohort stock of *T. pacificus* but also due to changes in distribution (Hokkaido Research Organization, 2024). Overfishing in the northern Japan Sea (outside the Exclusive Economic Zone of Japan) also possibly caused the decline (Park *et al.*, 2020). To clarify the mechanism of distribution changes, it is necessary to investigate more specifically the migration routes of the squid.

Biologging and biotelemetry methods have been developed to improve understanding of migratory routes and behaviour of marine organisms that grow to moderate or large sizes but such methods are still difficult to apply to rather small or physically fragile organisms including *T. pacificus*.

As a possible archive of environmental conditions experienced by an individual, elemental composition of biogenic carbonates precipitated by marine organisms has been studied intensively. In particular, strontium (Sr) is the most useful element because it is incorporated into aragonitic structures at a relatively high concentration and often reflects environmental conditions in many species (reviewed by Doubleday *et al.*, 2014; Avigliano *et al.*, 2020). The strontium-to-calcium ratio (Sr:Ca) deposited in aragonitic structures has been shown to represent a record of the temperatures experienced by an individual in some species (e.g. Schöne *et al.*, 2011; Shirai *et al.*, 2014 for the bivalve *Arctica islandica*); and otolith Sr:Ca has often been used for estimating the age at which diadromous fish species make the transition between marine and freshwater habitats (e.g. Murase *et al.*, 2019). In gastropods and bivalves, the relationship between temperature and shell Sr:Ca is usually reported to be negative (Schöne *et al.*, 2011; Füllenbach *et al.*, 2015), but it may be positive (Wanamaker *et al.*, 2008; Irie and Suzuki, 2020), and sometimes there may be no clear relationship (Vander Putten *et al.*, 2000), depending on species. In squids, the statolith is the only calcified structure allowing the Sr:Ca-based reconstruction of age-associated environmental temperatures (Ikeda *et al.*, 2003; Liu *et al.*, 2016; Jones *et al.*, 2018), because daily increment periodicity in statolith growth enables the estimation of individual age by counting growth rings (reviewed by Jackson, 2004). Ikeda *et al.* (2003) suggested that the negative relationship of statolith Sr:Ca and water temperature could be used to monitor migratory behaviour of *T. pacificus* captured at sea. Yamaguchi *et al.* (2015, 2018) estimated the migratory routes of *Uroteuthis edulis* after an experimental evaluation of the negative relationship between statolith Sr:Ca and water temperature. Recently, Hosono *et al.* (2022) have also shown a similar negative relationship through rearing experiments with *Heterololigo bleekeri*. However, without a large laboratory rearing tank under controlled temperature, keeping squid under good conditions is difficult even for a short period (Hanlon, 1990; Takahara *et al.*, 2017).

The approach of the present study is to use a regional ocean model and Lagrangian tracer experiments with the aim of evaluating the quantitative relationship between empirical water temperature and statolith Sr:Ca to improve our understanding of the migration of *T. pacificus*. Although *T. pacificus* individuals can swim southwestwards in the Japan Sea against the Tsushima Warm Current in the last half of their lifetime (Kidokoro *et al.*, 2010), it is considered that, in the first half, they move northeastwards, probably making the best use of the Tsushima Warm Current. Therefore, micro-increments and Sr:Ca were investigated in the statoliths of immature squid collected in July near the west coast of Hokkaido (their feeding area, in the northeastern Japan Sea). Water temperatures on the trajectory of Lagrangian backward tracers were calculated from the collection site and date.

Materials and methods

Squid samples

A total of 33 specimens of *Todarodes pacificus* were collected at three stations: near the west coast of Hokkaido Island (WH) on 6th July 2020; east of Tsushima Island (ET) on 15th January 2021; and west of the Goto Islands (WG) on 16th January 2021. Biological data and sampling locations are shown in Table 1 and Figure 1.

Micro-increment counts

After removal of the right and left statoliths from all the samples, each was cleaned with 95% ethanol, embedded in Stickwax (Maruto Instrument Ltd., Tokyo, Japan), and mounted on a glass slide with the anterior side upwards. After hardening, the right statolith was ground along the horizontal plane until the nucleus was exposed. It was then turned over and attached to the glass slide with the posterior side up and ground again to the nucleus. Fine-grade waterproof carbide sandpapers and lapping film sheets were used to grind the statolith, and a thin section of the statolith in the lateral plane was obtained for micro-increment counts (Arkhipkin and Shcherbich, 2012).

The counting of the increments was carried out using a light microscope with a monitor, resulting in a final magnification of 500×. The number of increments on each statolith was counted from the nucleus to the edge of the dorsal dome. Counting of increments was performed three times and the mean value was adopted if the range of counts was within 10% of the mean, and if >10% the statoliths were read again until the range of counts was within 10% of the mean, as determined by a single observer. The hatching date of each squid was estimated from the number of increments and the date of capture, assuming daily deposition of increments (Nakamura and Sakurai, 1991).

Sr and Ca measurements

The left statoliths from all 33 specimens were prepared for Sr and Ca measurements according to the method of Yamaguchi *et al.* (2015). Each statolith was cleaned, dried, and mounted on a glass slide in a cylindrical silicone stub filled with acrylic resin (Shofu Ortho Palette; SHOFU Inc., Japan) with the anterior side down. After hardening, the polyester block was ground from the anterior side with fine-grade carbide waterproof sandpaper until the nucleus appeared as close to the surface as possible (i.e. the nucleus was not always exposed). Ground statoliths were polished with 3 μm diamond paste before Sr and Ca measurements.

Line analysis was performed to understand the variation in the ratio of Sr and Ca contents in the 33 statoliths using an electron

Table 1. Collection data of *Todarodes pacificus* specimens sampled for this study

					Mantle length (mm)		Body weight (g)			Age (day)		Hatching date
Collection date	Collection site		Sex	Number	Mean ± SD		Mean ± SD		Maturation	Mean ± SD		
6 July 2020	43.50N	140.68E	F	4	171	10	86	20	Immature	188	21	1 January 2020
	WH		M	4	169	12	87	19	Immature			
15 January 2021	34.57N	129.63E	F	4	272	10	471	41	Mature	264	21	26 April 2020
	ET		M	6	242	15	332	46	Mature			
16 January 2021	32.75N	128.25E	F	5	277	14	491	85	Mature	268	15	23 April 2020
	WG		M	10	252	19	373	87	Mature			

WH, off the west coast of Hokkaido Island; ET, east of Tsushima Island; WG, west of the Goto Islands; F, female; M, male; SD, standard deviation.

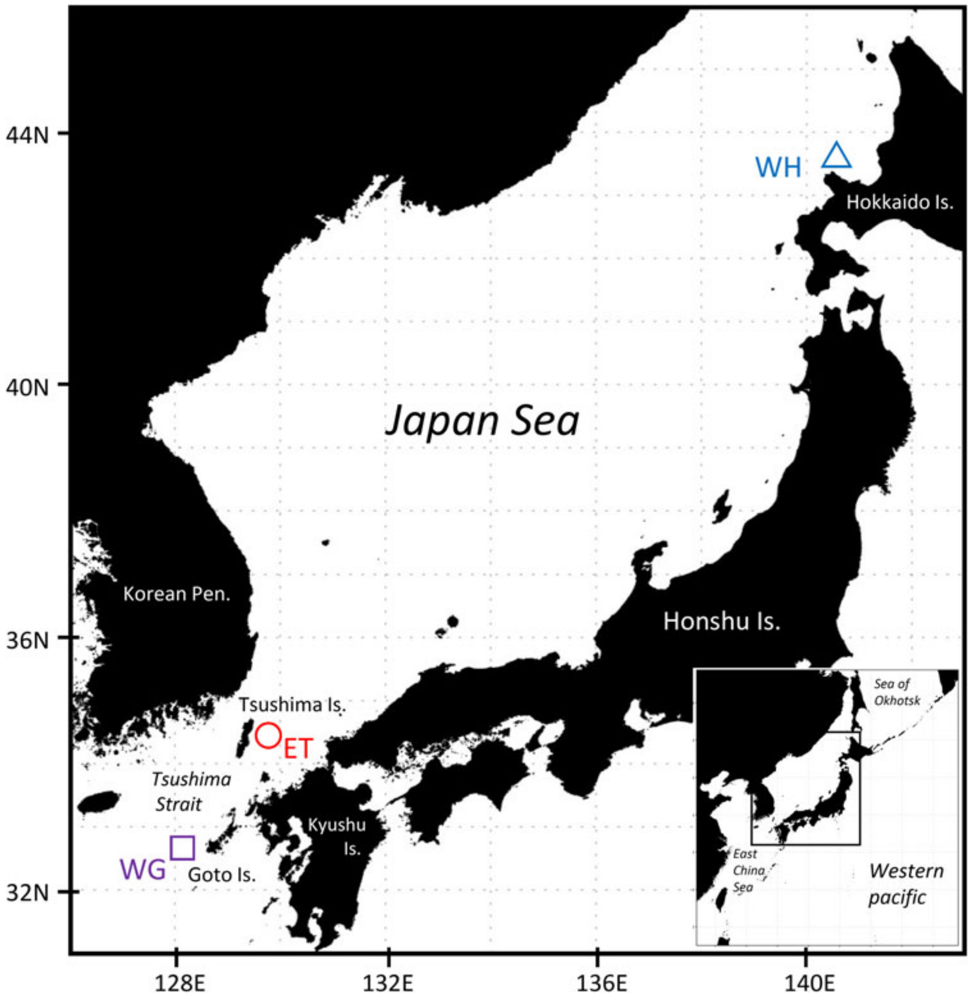

Figure 1. Map of the study area. The three *Todarodes pacificus* collection sites are indicated with a triangle (off western Hokkaido, WH), a circle (east of Tsushima Island, ET), and a square (west of the Goto Islands, WG).

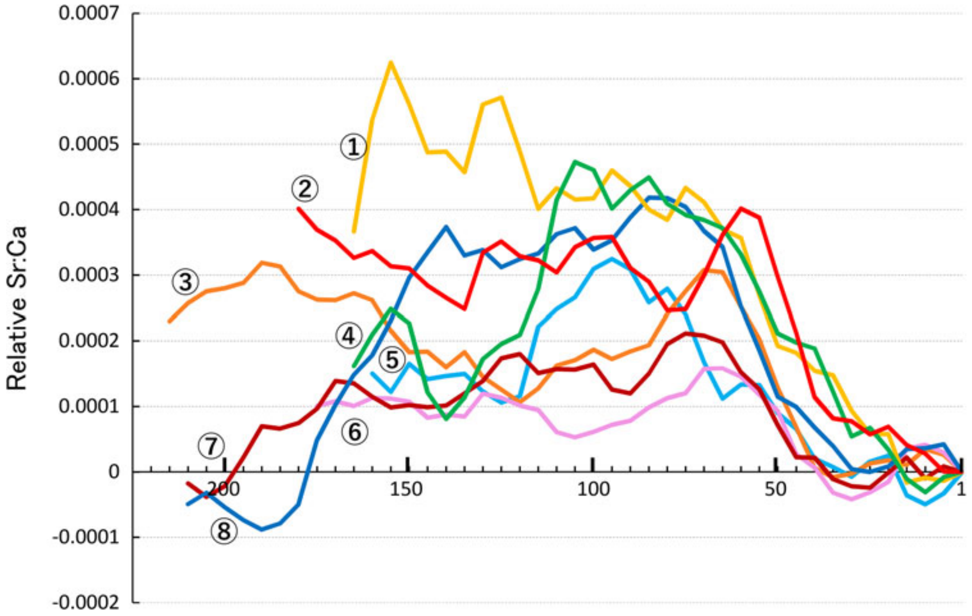

Figure 2. Lifetime variation of Sr:Ca in the statoliths of the eight *T. pacificus* specimens (①–⑧) collected at WH on 6 July 2020. Ordinate: Sr:Ca ratio relative to the value obtained at date of capture. Abscissa: Days before collection date.

probe microanalyzer (EPMA, JXA-8530F; Jeol Ltd.) installed at Kyushu University. The analytical conditions were as follows: acceleration voltage 10 kV, beam current 100 nA, and beam diameter 5 µm. Sr-Kα and Ca-Kα line intensities were acquired by analysing crystals of thallium acid phthalate and phosphorus electron-transparent heterogeneity, respectively. Measurements

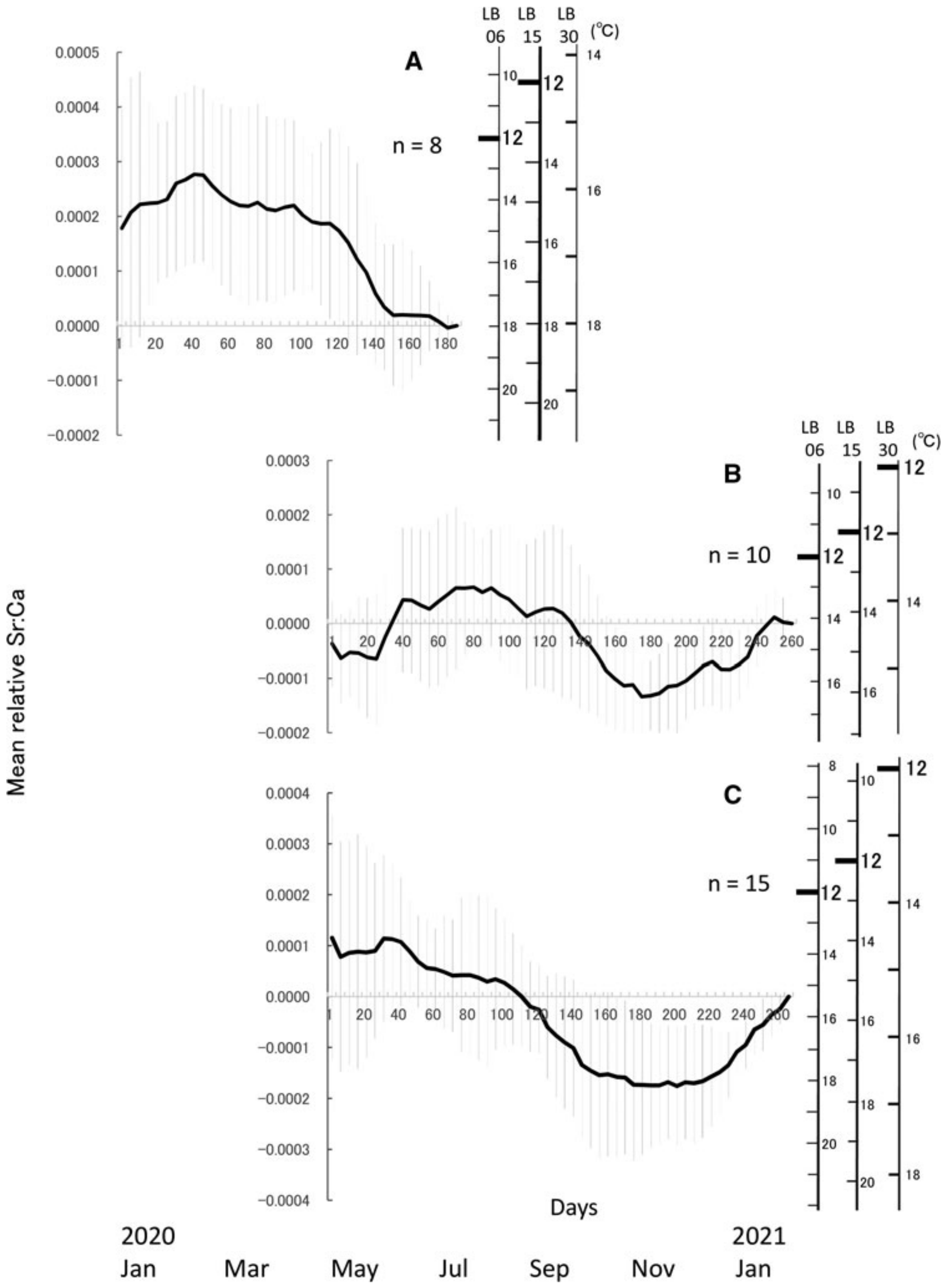

Figure 3. Mean relative Sr:Ca variation (±SDs) of *T. pacificus* collected at WH, ET, and WG. Left ordinate: Sr:Ca relative to that at date of capture. Right ordinates: empirical water temperature estimated through regression expressions for the SW group using Lagrangian backward tracer depth experiments LB06, LB15, and LB30. The lower limit of the water temperature appropriate for *T. pacificus* (12°C) is indicated in bold. Note that the scales are such that temperature increases downwards, towards the foot of the page. Abscissa: Days since estimated hatching date.

were performed from the nucleus to the lateral dome edge of the statolith at measurement intervals of 5 μm and a counting time of 20 s for each spot.

A qualitative analysis of a single squid statolith was conducted using the same analytical conditions as the line analysis to obtain background counts, which were found to be approximately 3500 for Sr and 5500 for Ca. These values were subtracted from the raw intensity (gross counts) of the signal from each statolith measurement and the background and he ratio of Sr and Ca obtained using these values was defined as Sr:Ca.

After measuring the trace elements from the nucleus to the edge of each statolith, the Sr:Ca values were calculated and extracted at 5-day intervals from the hatching date, taking into account the daily age of each squid, assuming that statolith micro-increments were formed at even intervals. The relative change from the hatching date was calculated using the value on the catch date as the standard to cancel the individual differences: this is because it is known from research on *Uroteuthis edulis* (swordtip squid) that Sr:Ca of several individuals maintained at the same water temperature did not match exactly although the variation trends were similar (Yamaguchi *et al.*, 2015). Subsequently, 5-day averages were calculated for each sampling area.

Lagrangian backward tracer experiments

The ocean environment was calculated using the high-resolution data assimilation outputs of water temperature and currents produced by Hirose *et al.* (2013). Prognostic time integration was modelled by the Research Institute for Applied Mechanics (RIAM) Ocean Model (DREAMS at RIAM [kyushu-u.ac.jp]),

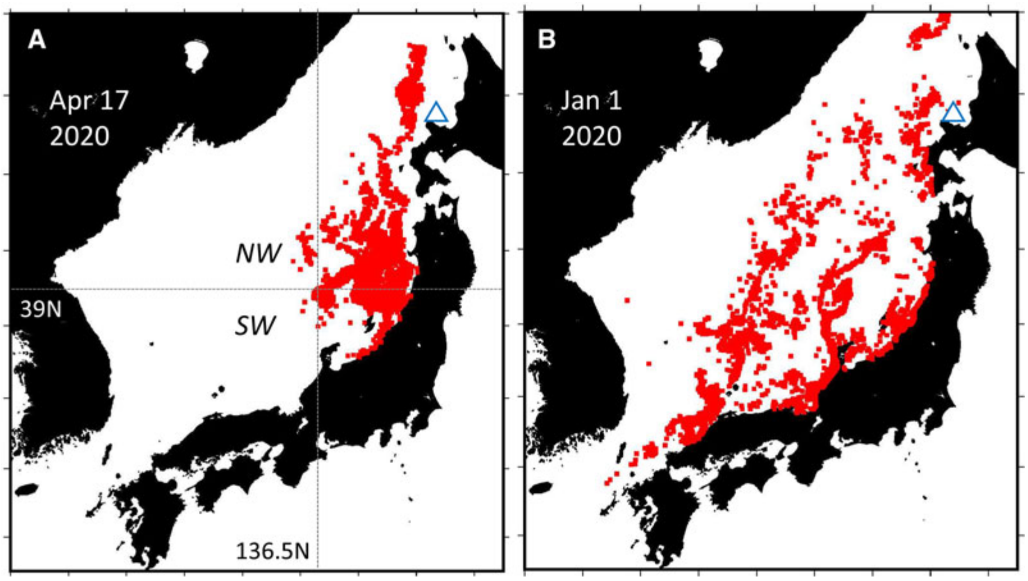

Figure 4. Distributions of Lagrangian backward tracers on (A) 17 April and (B) 1 January 2020 for LB15. The release point is indicated by the triangle (location of WH sampling). *Todarodes pacificus* were identified to the west of 136.5°E and the south of 39°N (SW quadrant) around 17 April 2020 through a recruitment survey.

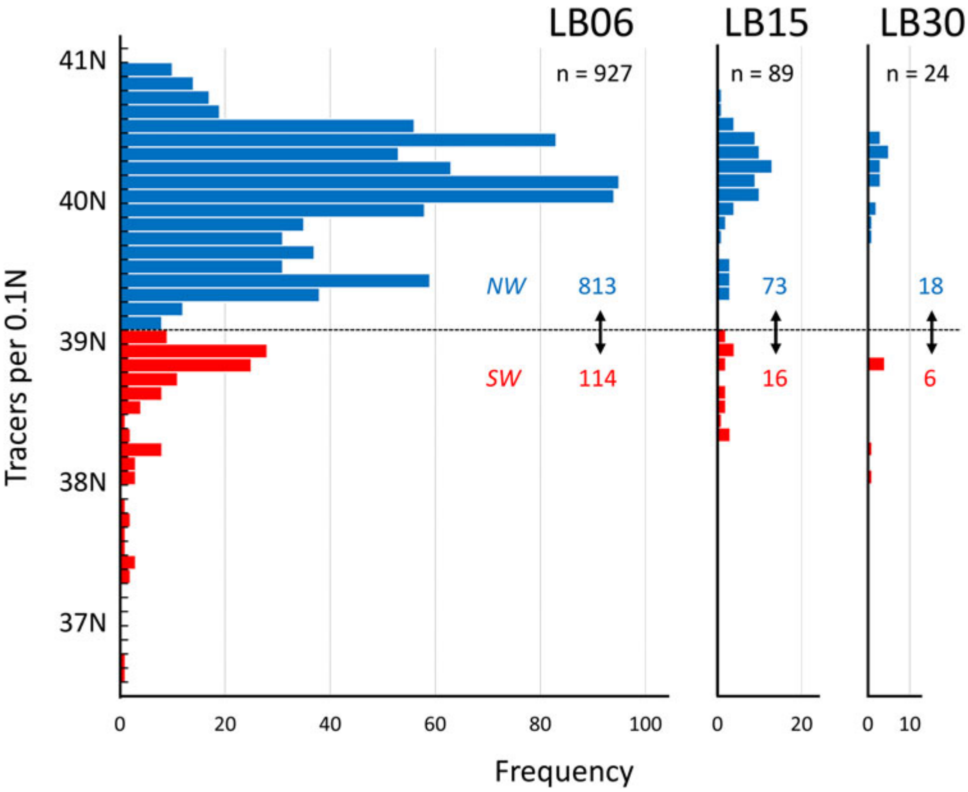

Figure 5. Histogram of the location of Lagrangian backward tracers that reached further west than 136.5°E on 17 April (SW and NW) for experiments LB06, LB15, and LB30.

which is a hydrostatic, *z* coordinate, and primitive-equation ocean general circulation model. The spherical coordinate model, with a resolution of 1/12° longitude and 1/15° latitude, covers both the East China Sea and the Japan Sea. Diagnostic analysis, with an approximate Kalman filter, sequentially corrected the modelled variables by assimilating the remotely-sensed surface temperature and height data with *in situ* temperature and salinity profiles.

In the backward tracer experiments, 10,000 Lagrangian tracers were released at 43.50°N, 140.68°E (WH) on 6 July 2020, the location and date of capture of eight immature specimens (Figure 1). Tracer advections and diffusions were calculated using the method of Kato *et al.* (2014). The Smagorinsky coefficient was set at 0.1.

For these backward tracer experiments, the squid were assumed to move passively with the ocean currents and the tracers were set to move continuously in the exact opposite direction of the ocean current. There is very little information about vertical migrations of squid, so previous numerical experiments have assumed constant depths of the passive tracers based on an appropriate water temperature range for this species (Fujii *et al.*, 2004). In the present study, an appropriate depth for the tracers was determined based

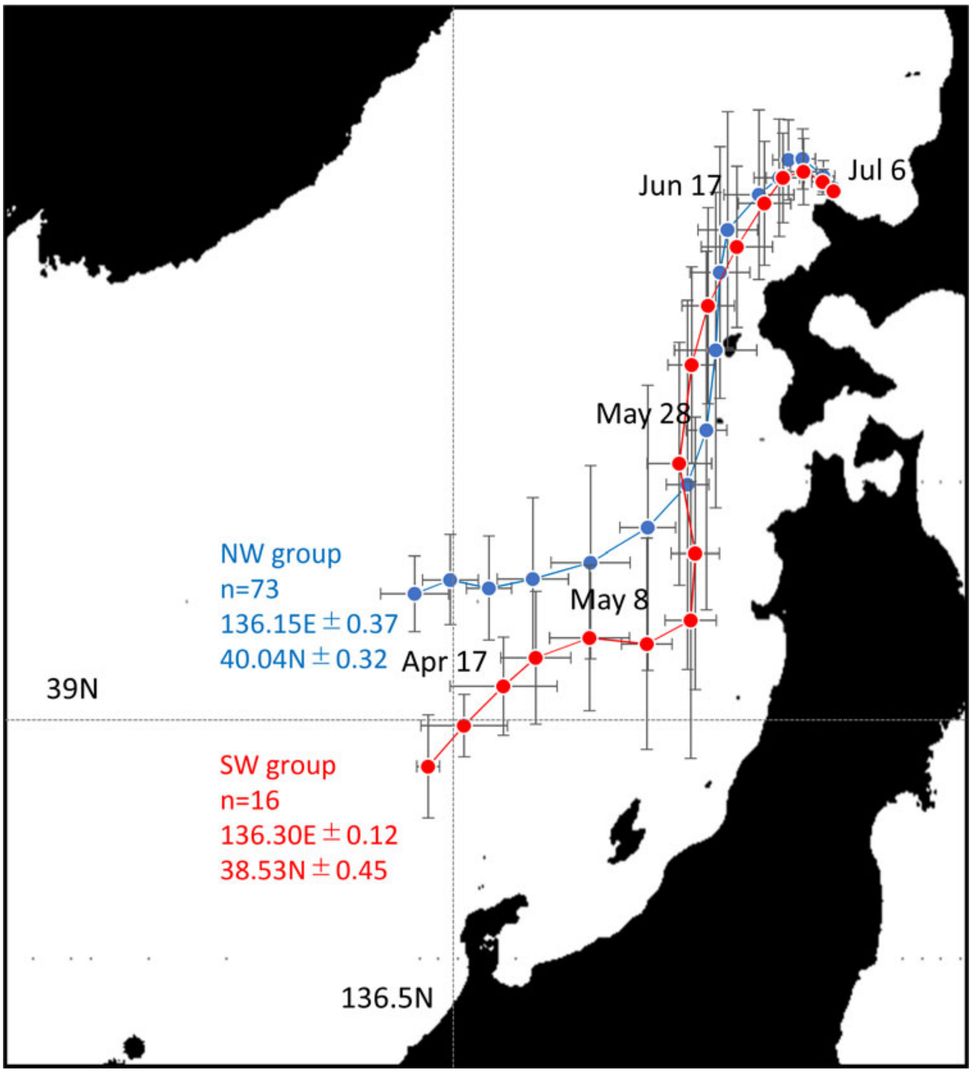

Figure 6. Five-day interval median coordinates (±SDs) of Lagrangian backward tracers that reached further west than 136.5°E and south (SW) and the north (NW) of 39°N on 17 April 2020, with LB15.

on the results of comparative experiments. The results were evaluated using the data from Sr:Ca and water temperatures in the northeastern Japan Sea for the first half of the squid lifetime.

Lagrangian backward (LB) tracer experiments were conducted at depths of 6 m (LB06), 15 m (LB15), and 30 m (LB30). Only the tracers that reached south of 39°N and west of 136.5°E (SW) in the Japan Sea were extracted as migrating squid data. This is because the species was identified in that region during a recruitment survey conducted by the Fisheries Agency of Japan and the Japan Fisheries Research and Education Agency from 14 to 20 April 2020 (Kubota *et al.*, 2021). The survey was carried out at 33 locations east of 133.5°E and south of 39°N, but squid were collected only at survey sites located west of 136.5°E. Although recruitment surveys were not conducted north of around 39°N, the tracers to the north of 39°N and west of 136.5°E (NW) on 17 April 2020, were also found to be useful. Preliminary experiments showed very few or no tracers in the above target areas on 17 April 2020, at a depth of 50 m or deeper (data not shown).

Lagrangian forward tracer experiments

Lagrangian forward tracer experiments were conducted at a depth of 15 m from 1 November 2019, and from 1 January 2020, using the same conditions as those for the Lagrangian backward tracer experiments. The release point was in the eastern Tsushima Strait (33.5°N, 129°E). The number of paralarvae caught in autumn research surveys in the eastern Tsushima Strait has recently been fewer than during the 1990s (Kubota *et al.*, 2021). To examine the fate of the lower number of paralarvae, Lagrangian forward tracer experiments were conducted at a depth of 15 m from 1 November 2019, and from 1 January 2020. The release point was in the eastern Tsushima Strait (33.5°N, 129°E).

Water temperature estimation along the migration trajectory

The median points of the Lagrangian tracers that reached the SW Japan Sea on 17 April 2020 were calculated backwards every 5 days from the release day (SW group), and then water temperature at each point was determined using DREAMS_M. Those reaching the NW coordinates on 17 April 2020 (NW group) also were calculated and each water temperature was determined. The correlation between the mean Sr:Ca of the eight squid statoliths and the water temperatures of the SW and NW groups was estimated through the backward tracer experiments of LB06, LB15, and LB30 and regression expressions were calculated.

The water temperature variations experienced by *T. pacificus* collected at the three sampling locations were estimated through regression expressions that relate the Sr:Ca data to the water temperatures of the SW and NW groups across the three LB experiments. To evaluate the validity of these estimated water

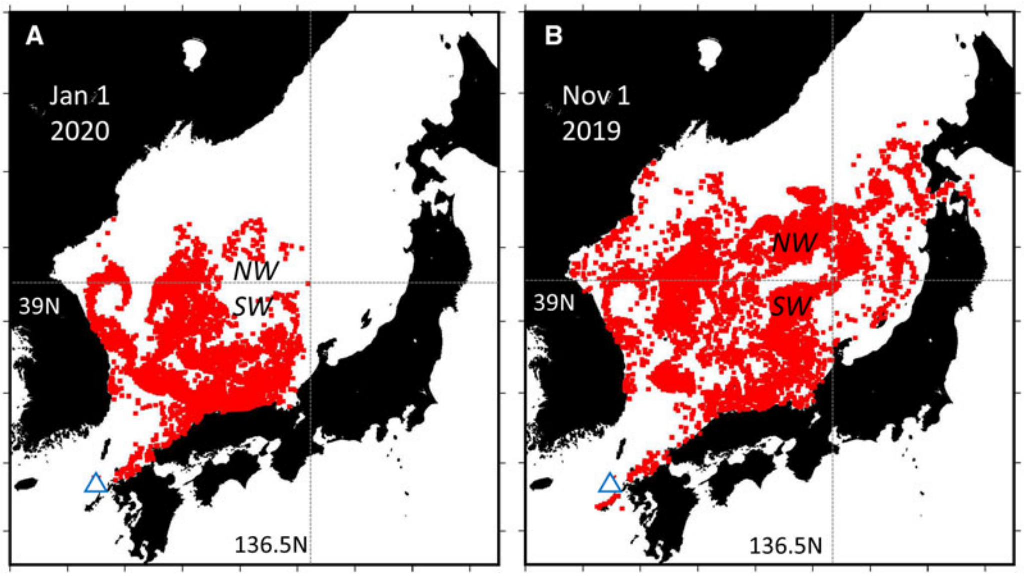

Figure 7. Distributions of Lagrangian forward tracers on 17 April 2020, released at a depth of 15 m on (A) 1 January 2020 and (B) 1 November 2019. The release point is indicated by the triangle in the eastern Tsushima Strait (33.5°N, 129°E).

temperature variations, they were assessed as a proportion of the appropriate habitat water temperature range known for this species (12–23°C; Sakurai *et al*., 2013) and expressed as an appropriate temperature (AT) ratio.

Results

Age at date of capture and estimated hatching date

The age at capture and estimated mean date of hatching for the three sampling areas are listed in Table 1. The squids captured to the west of Hokkaido (WH) in July had hatched about 6 months earlier, while the ET and WG Japan Sea groups caught in January were about 9 months old and had hatched in April the previous year.

Variations of Sr:Ca in statoliths

The mean numbers and standard deviations (SDs) of Sr:Ca measurement points from the nucleus to the lateral dome edge in statoliths of the three groups of sampled squids were 71 ± 9 (WH), 82 ± 13 (ET), and 86 ± 9 (WG). However, even among individuals collected at the same station, their daily ages varied slightly. Additionally, the number of points where Sr and Ca were measured in the statolith did not necessarily correspond to the daily age. Therefore, to represent the Sr:Ca variation relative to the average daily age, the Sr:Ca values for each squid were extracted at 5-day intervals. Consequently, the mean numbers of measurements were 38 (WH), 53 (ET), and 54 (WG).

Changes in Sr:Ca from the centre (hatching date) to the edge (captured date) of the statolith for each of the eight squid captured at WH are shown in Figure 2, and the mean Sr:Ca are shown in Figure 3 for each of the WH, ET, and WG groups. The mean Sr:Ca of the immature squids collected at WH on 6 July (Figure 3A) remained high until the 120th day after hatching (around May) and then declined towards zero around the time of capture. For the mature squids caught at ET on 15 January (Figure 3B), the mean Sr:Ca was positive between the 40th and 130th day after hatching (around June to September), and thereafter was negative until the 180th day (around November), becoming positive again by the day of capture. The changes in mean Sr:Ca for the WG group, captured on 16 January (Figure 3C) decreased steadily from the hatching date to just before the 120th day, becoming negative until the 190th day (around November), just before capture.

Lagrangian backward tracer experiments

The Lagrangian backward tracers released from WH on 6 July 2020, generally moved back southwestwards in the Japan Sea, and some tracers reached the Tsushima Strait before 1 January 2020 (the estimated hatching date of the squid collected at WH on 6 July 2020; Figure 4). The distribution of tracers on 17 April 2020 is shown in Figure 5. The trajectories of the SW and

Table 2. Correlation coefficients between relative Sr:Ca ratios in statoliths of *T. pacificus* (specimens ①–⑧) collected off the west coast of Hokkaido and ambient water temperatures of the SW and NW groups calculated from Lagrangian backward tracer experiments at three different depths LB06, LB15, and LB30 (see section 'Materials and methods' for details)

Depth	Group	①	②	③	④	⑤	⑥	⑦	⑧	Mean
LB06	NW	−0.927	−0.802	−0.794	−0.940	−0.849	−0.644	−0.800	−0.831	−0.882
	SW	−0.932	−0.886	−0.832	−0.941	−0.807	−0.726	−0.825	−0.826	−0.904
LB15	NW	−0.895	−0.756	−0.792	−0.910	−0.816	−0.636	−0.795	−0.841	−0.862
	SW	−0.915	−0.911	−0.880	−0.926	−0.800	−0.795	−0.852	−0.838	−0.917
LB30	NW	−0.911	−0.730	−0.778	−0.896	−0.937	−0.650	−0.817	−0.858	−0.874
	SW	−0.921	−0.776	−0.772	−0.923	−0.895	−0.626	−0.789	−0.821	−0.872

NW groups of LB15 are shown in Figure 6 (data not shown for trajectories of LB06 and LB30 since they were little different from that of LB15).

Lagrangian forward tracer experiments

The result of the forward tracer experiment from 1 January 2020 showed the easternmost tracer reached just west of 136.5°E on 17 April 2020 (Figure 7A), corresponding to the location of the westernmost tracer with the LB15 experiment (Figure 4A). Meanwhile, many forward tracers released on 1 November 2019, advected to the east of 136.5°E by 17 April 2020 (Figure 7B).

Relationship between Sr:Ca and water temperatures

The correlations between the individual and mean Sr:Ca in the statoliths of the eight immature squid collected at WH on 6 July 2020 and water temperatures at the median points of trajectories of the SW and NW groups on LB06, LB15, and LB30 were assessed (the three LBs *vs* the two geographical groups, for a total of six comparisons; Table 2). The strongest negative correlation between the mean Sr:Ca and water temperature was in the SW group for LB15 ($r = -0.917$), and the second strongest in the SW group for LB06 ($r = -0.904$).

The regression expressions of relationships between the 17 mean Sr:Ca and 17 ambient water temperatures of the same days of the SW and NW groups on LB experiments LB06, LB15, and LB308 (Figure 8) are plotted in Figure 9. The strongest determination coefficient of the six regression expressions was in the SW group for LB15 ($R^2 = 0.841$).

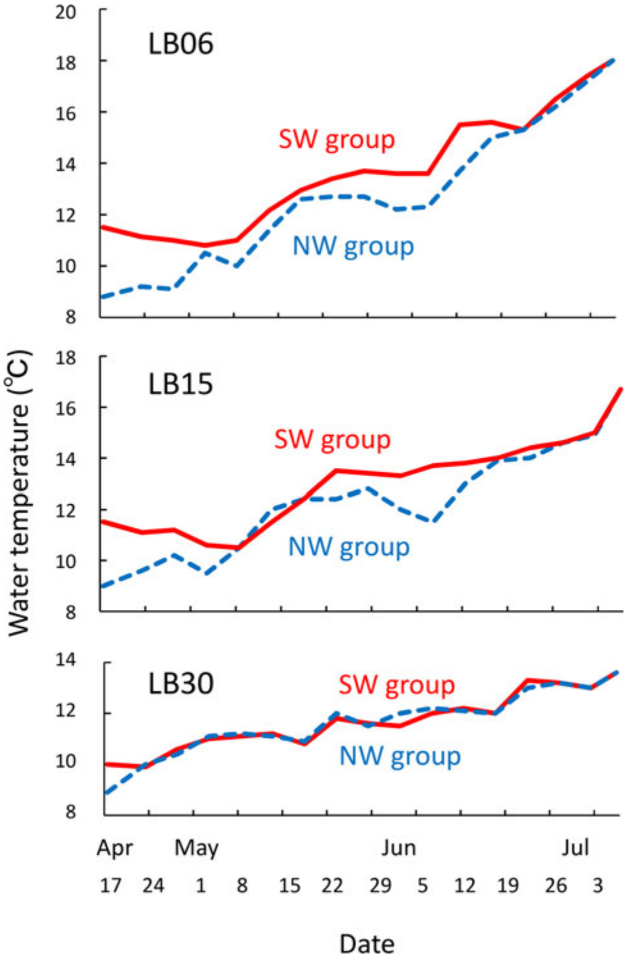

Figure 8. Ambient water temperature variations of the 5-day-interval median points of the SW and NW groups with Lagrangian backward tracer experiments LB06, LB15, and LB30.

Estimation of water temperature variations experienced by the squid

Using the proportionality factors for each regression expression of the six conditions in Figure 9, the empirical variations in lifetime water temperature of the squids were estimated (see the extrapolated temperatures of the six ordinates to the right in Figure 3): the estimated water temperatures differed depending on the proportionality factors of the regression expressions and the water temperatures when and where the squid were caught. The highest AT ratio was observed with LB30, and to some extent with LB15 (Table 3).

In the SW group of LB15 (whose regression expression has the strongest determination coefficient), the estimated mean empirical water temperature experienced by the immature squids collected at WH was between 13 and 15°C from January to May, then gradually increased, reaching 17–18°C during May and June (Figure 3A). The water temperature experienced on the collection date by the 10 mature ET squid was estimated to be 14.2°C, and by the 15 mature WG squid to be 15.2°C. The vertical profile of the water temperature in the Tsushima Strait was almost uniform from the surface to a depth of about 150 m from autumn to spring because of seasonal vertical mixing (Shibano *et al.*, 2019). The mean empirical water temperature variation of the ET squid remained between 13 and 14°C from May to September after a steady decrease from 15°C as juveniles (Figure 3B). Then, the variation increased to 16°C in October and decreased until January. The mean empirical water temperature variation of the 15 squid collected at WG remained more or less between 14 and 15°C from April to August and increased to 18°C in November before a decrease until January (Figure 3C).

To estimate water temperatures the squid were experienced accurately, the most reasonable combination of the six conditions was determined through four grades, A(1) > B(2) > C(2) > D(1), where each numerical value of its grade shows the number of its grade. Overall, the SW group with the LB15 simulation showed the strongest negative correlation (between the mean statolith Sr:Ca and water temperatures at the median points of the tracer trajectories; Table 2), and the third best AT ratio after the SW and NW groups on LB30 (Table 4). For the SW and NW groups with LB30, relatively very few Lagrangian tracers appeared on 17 April 2020. Moreover, the negative correlations and AT ratios of the SW and NW groups with LB06 were lower than those of the SW group on LB15. Therefore, of the six combinations, the regression expression for the SW group with LB15 provided the best estimate of the lifetime empirical water temperature variation of the squid.

Discussion

As a result of the statolith analyses and Lagrangian tracer experiments, the best combination out of the six LB simulations was the condition of the SW group on LB15, and the second best was that of the NW group on LB06 (Table 4). Considering what is known of the ecology of this species (Sakurai *et al.*, 2013), the fixed depth of 15 m on the tracer experiment is reasonable, although the squid probably undergo diurnal migrations (Kawabata *et al.*, 2006). Figure 8 suggests that, from April to July, the water temperatures in the northeastern Japan Sea below a depth of 30 m were probably 13°C or lower. The appropriate water temperature range of this species is reported to be between 12 and 23°C (Sakurai *et al.*, 2013), so a mean depth of 15 m is consistent with its known normal temperature preference range.

Table 3. Estimated AT ratios (%) (ratio of empirical water temperatures lying within appropriate water temperatures of 12–23°C; see final section of 'Materials and methods') for *T. pacificus* collected at each of the three sampling sites (WH, ET, and WG), as estimated through six regression expressions (the SW and NW groups *vs* the three Lagrangian backward tracer depths LB06, LB15 and LB30)

Regression expression	SW group			NW group		
Collection site	WH	ET	WG	WH	ET	WG
LB06	81.5	89.4	91.7	71.9	86.6	86.6
LB15	94.9	92.8	95.8	88.8	90.3	93.9
LB30	100.0	96.7	98.6	100.0	96.2	98.3

Table 4. Score sheet for six combinations of three experiments and two groups across three results of analyses

Experiment	LB06		LB15		LB30	
Group	SW	NW	SW	NW	SW	NW
Number of backward tracers	B	A	C	B	D	C
Determination coefficient	B	B	A	D	C	C
AT ratio	C	D	B	C	A	B

Grade (the number of each grade), A(1) > B(2) > C(2) > D(1).

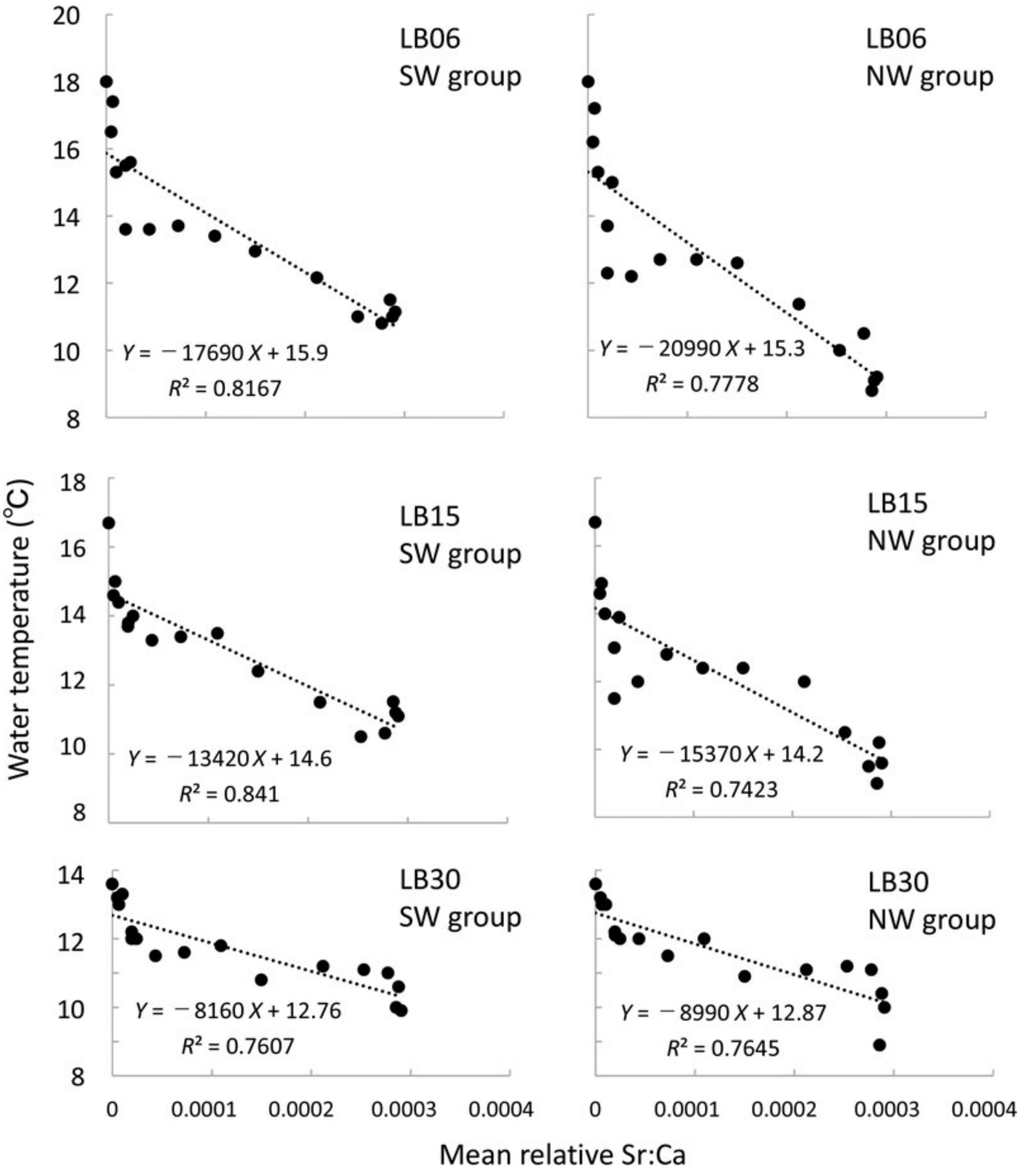

Figure 9. Regression lines and equations (with determination coefficient) for mean relative Sr:Ca in statoliths of *T. pacificus vs* ambient water temperatures of the SW and NW groups with Lagrangian backward tracer experiments LB06, LB15, and LB30.

Therefore, the Sr:Ca in the statoliths of *Todarodes pacificus* species showed a negative correlation with empirical water temperatures in the present study, consistent with the findings of Ikeda *et al.* (2003). Furthermore, a quantitative relationship between the Sr:Ca variation and empirical water temperatures for this species was established for the first time.

The temperature variations obtained from statolith Sr:Ca variations of the squid collected in the Tsushima Strait in January generally corresponded to the known seasonal variation of seawater temperature in the sub-surface layer of the southern Japan Sea and Tsushima Strait from summer to winter (online Figure S1). Although the highest sea temperature is usually during September, the squid collected at ET and WG experienced their highest water temperature in October and November, respectively. This time lag suggests that the squid had possibly moved towards the warmer waters, which is consistent with a hypothesis explaining the spawning migration from the Japan Sea to the Tsushima Strait (Kidokoro *et al.*, 2010; Rosa *et al.*, 2011). The results for long-lived squid also clearly indicated that the statolith Sr:Ca variation was affected by the empirical water temperatures.

Thus, the migratory route of *T. pacificus* can be determined using Lagrangian tracer experiments, assuming that ocean currents influence the migration, at least during its early life stages. This method also has the potential to reveal the relationship between the hatching grounds and distant fishing grounds. A squid catches of round 400 kg in the eastern Tsushima Strait were reported for this week (Nagasaki Prefecture Local Government, 2020) on January 2020. The result of this experiment showed the easternmost tracer reached just west of 136.5° E on 17 April (Figure 7A), corresponding to the location of the westernmost tracer with the LB15 experiment (Figure 4A). Meanwhile, many forward tracers released on 1 November 2019 advected to the east of 136.5°E by 17 April 2020 (Figure 7B). If a larger spawning population had been produced in autumn (as occurred a few decades ago), it is expected that more squid would have approached WH in the following July. In reality, the number of squid present around western Hokkaido has been decreasing catastrophically. These results suggest that the presence of fewer paralarvae in the eastern Tsushima Strait in autumn is one cause of the recent poor catches around western Hokkaido in the following summer, which marks the first success in elucidating the relationship between the hatching grounds and the fishing grounds through the use of a numerical ocean model.

In conclusion, this study successfully demonstrated the efficacy of using a combination of interdisciplinary methods to achieve a better understanding of some of the main features determining the migration of *T. pacificus*. However, further sampling of squids in the field is required to confirm their migratory routes.

Supplementary Material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315425000098.

Data. The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Acknowledgements. We thank Dr Tatsuro Adachi for providing technical advice during the EPMA analysis; and Mr Kenichiro Omote of Marino Research Co., Ltd, for his cooperation during the statolith increment analysis.

Author Contributions. T. Yamaguchi performed statolith Sr:Ca measurements, Lagrangian tracer experiments, data analyses, and writing. H. Matsui, H. Miyahara, and H. Kubota collected and measured squids. N. Hirose managed the regional ocean model and revised the manuscript.

Financial support. This study was supported by a grant from the Research and Assessment Program for Fisheries Resources, the Fisheries Agency of Japan to H. Matsui, H. Miyahara, and H. Kubota and the Nakatsuji Foresight Foundation Research Grant to T. Yamaguchi.

Competing interests. None.

Ethical standards. All procedures performed in the present study followed the ethical standards of the Life Science Research Ethics.

References

Arkhipkin AI, Rodhouse PGK, Pierce GJ, Sauer W, Sakai M, Allcock L, Arguelles J, Bower JR, Castillo G, Ceriola L, CS Chen, X Chen, M Diaz-Santana, N Downey, AF González, JG Amores, CP Green, A Guerra, LC Hendrickson, C Ibáñez, K Ito, P Jereb, Y Kato, ON Katugin, M Kawano, H Kidokoro, VV Kulik, VV Laptikhovsky, MR Lipinski, B Liu, L Mariátegui, W Marin, A Medina, K Miki, k Miyahara, N Moltschaniwskyj, H Moustahfid, J Nabhitabhata, N Nanjo, CM Nigmatullin, T Ohtani, G Pecl, JAA Perez, U Piatkowski, P Saikliang, CA Salinas-Zavala, M Steer, Y Tian, Y Ueta, D Vijai, T Wakabayashi, T Yamaguchi, C Yamashiro, N Yamashita and LD Zeidberg (2015) World squid fisheries. Reviews in Fisheries Science and Aquaculture 23, 92–252.

Arkhipkin AI and Shcherbich ZN (2012) Thirty years' progress in age determination of squid using statoliths. *Journal of the Marine Biological Association of the United Kingdom* **92**, 1389–1398.

Avigliano E, Volpedo AV and Walther BD (2020) Editorial: Studying the biology of aquatic animals through calcified structures. *Frontiers in Marine Science* **7**, Article 687, 1–3.

Doubleday ZA, Harris HH, Izzo C and Gillanders BM (2014) Strontium randomly substituting for calcium in fish otolith aragonite. *Analytical Chemistry* **86**, 865–869.

Fujii Y, Hirose N, Watanabe T and Kidokoro H (2004) Numerical simulation of the transport for eggs and larvae of *Todarodesu pacificus* steenstrup (Surume-ika) in the Japan Sea. *Journal of the Meteorological Society of Japan* **80**, 9–17 (in Japanese with English abstract.).

Füllenbach CS, Schöne BR and Mertz-Kraus R (2015) Strontium/lithium ratio in aragonitic shells of *Cerastoderma edule* (Bivalvia) – a new potential temperature proxy for brackish environments. *Chemical Geology* **417**, 341–355.

Gleadall IG, Moustahfid H, Sauer WHH, Ababouch L, Arkhipkin AI, Bensbai J, Elegbede I, Faraj A, Ferreiro-Velasco P, González-Gómez R, González-Vallés C, Markaida U, Morillo-Velarde PS, Pierce GJ, Pirro S, Pita C, Roumbedakis K, Sakurai Y, Scheel D, Shaw PW, Veiga P, Willette DA, Winter A and Yamaguchi T (2024) Towards global traceability for sustainable cephalopod seafood. *Marine Biology* **171**, 44.

Goto T (2002) Paralarval distribution of the ommastrephid squid *Todarodes pacificus* during fall in the southern Sea of Japan and its implication for locating spawning grounds. *Bulletin of Marine Science* **71**, 299–312.

Hanlon RT (1990) Maintenance, rearing, and culture of teuthoid and sepioid squids. In Gilbert DL, Adelman WJ and Arnold JM (eds), *Squid as Experimental Animals*. Boston, MA: Springer, pp. 3–62. https://doi.org/10.1007/978-1-4899-2489-6_4.

Hirose N, Takayama K and Moon JH (2013) Regional data assimilation system extended to the east Asian marginal seas. *Sea and Sky* **89**, 1–9.

Hokkaido Research Organization (2024) Japanese flying squid (in the Japan Sea). Stock assessment and evaluation for major fish species in the surrounding sea areas of Hokkaido (fiscal year 2021). 491–509. Available at https://www.hro.or.jp/upload/43860/2023HokkaidoStockAssessment.pdf (accessed 4 February 2024).

Hosono S, Irie T, Yamamoto J, Nakaya M, Sakurai Y, Kawamura T and Iwata Y (2022) Negative temperature dependence of statolith Sr/Ca and its intraspecific variability in experimentally maintained spear squid *Heterololigo bleekeri*. *Journal of the Marine Biological Association of the United Kingdom* **102**, 315–321.

Ikeda Y, Arai N, Kidokoro H and Sakamoto W (2003) Strontium: calcium ratios in statoliths of Japanese common squid *Todarodes pacificus* (Cephalopoda: Ommastrephidae) as indicators of migratory behavior. *Marine Ecology Progress Series* **251**, 169–179.

Irie T and Suzuki A (2020) High temperature stress does not distort the geo chemical thermometers based on biogenic calcium carbonate: stable oxygen isotope values and Sr/Ca ratios of gastropod shells in response to rearing temperature. *Geochimica et Cosmochimica Acta* **288**, 1–15.

Jackson GD (2004) Advances in defining the life histories of myopsid squid. *Marine and Freshwater Research* **55**, 357–365.

Jones JB, Arkhipkin AI, Marriott AL and Pierce GJ (2018) Using statolith elemental signatures to confirm ontogenetic migrations of the squid

Doryteuthis gahi around the Falkland Islands (southwest Atlantic). *Chemical Geology* **481**, 85–94.

Kato Y, Sakai M, Mmasujima M, Okazak M, Igarashi H, Masuda S and Awaji T (2014) Effects of hydrographic conditions on the transport of neon flying squid *Ommastrephes bartramii* larvae in the North Pacific Ocean. *Hidrobiologica* **24**, 33–38.

Kawabata A, Yatsu A, Ueno Y, Suyama S and Kurita Y (2006) Spatial distribution of the Japanese common squid, *Todarodes pacificus*, during its northward migration in the western North Pacific Ocean. *Fisheries Oceanography* **15**, 113–124.

Kidokoro H, Goto T, Nagasawa T, Nishida H, Akamine T and Sakurai Y (2010) Impacts of a climate regime shift on the migration of Japanese common squid (*Todarodes pacificus*). *ICES Journal of Marine Science* **67**, 1314–1322.

Kubota H, Miyahara H, Kaga T, Okamoto S, Nishijima S, Matsukura R, Tkasaki K, Saitoh T and Inagake D (2021) Stock assessment and evaluation for autumn-spawning stock of Japanese flying squid (fiscal year 2021). In *Marine Fisheries Stock Assessment and Evaluation for Japanese Waters*. Tokyo: Japan Fisheries Agency and Japan Fisheries Research and Education Agency, pp. 1–50. Available at https://www.fra.affrc.go.jp/shigen_hyoka/SCmeeting/2019-1/detail_surume-a_20201116.pdf (accessed 2 July 2023).

Liu BL, Cao J, Truesdell SB, Chen Y, Chen XJ and Tian SQ (2016) Reconstructing cephalopod migration with statolith elemental signatures: a case study using *Dosidicus gigas*. *Fisheries Science* **82**, 425–433.

Miyahara H, Okamoto S, Nishijima S, Matsukura R, Mstsui H, Moriyama T, Takasaki K, Saitoh T and Inagake D (2023) Stock assessment and evaluation for autumn-spawning stock of Japanese flying squid (fiscal year 2022). In *Marine Fisheries Stock Assessment and Evaluation for Japanese Waters*. Tokyo: Japan Fisheries Agency and Japan Fisheries Research and Education Agency, pp. 1–95. Available at https://www.fra.affrc.go.jp/shigen_hyoka/SCmeeting/2019-1/20221202/FRA-SA2022-SC11-02.pdf (accessed 1 November 2023).

Murase I, Kawakami T, Irie T and Iguchi K (2019) Counter-directional latitudinal clines of size at upstream migration between two adjacent water bodies in a Japanese amphidromous fish. *Marine Ecology Progress Series* **624**, 143–154.

Murata M (1989) Population assessment, management and fishery forecasting for the Japanese common squid, *Todarodes pacificus*. In Caddy JF (ed.), *Marine Invertebrate Fisheries: Their Assessment and Management*. New York: John Wiley & Sons, pp. 613–636.

Nagasaki Prefecture Local Government (2020) Weekly report of the fishery and oceanic conditions. Available at https://www.pref.nagasaki.jp/shared/uploads/2020/01/1578619210-1.pdf (accessed 3 December 2023).

Nakamura Y and Sakurai Y (1991) Validation of daily growth increments in statoliths of Japanese common squid *Todarodes pacificus*. *Nippon Suisan Gakkaishi* **57**, 2007–2011.

Okutani T (1983) *Todarodes pacificus*. In Boyle PR (ed.), *Cephalopod Life Cycles. 1. Species Accounts*. London: Academic Press, pp. 201–214.

Park J, Lee J, Seto K, Hochberg T, Wong BA, Miller NA, Takasaki K, Kubota H, Oozeki Y, Doshi S, Midzik M, Hanich Q, Sullivan B, Woods P and Kroodsma DA (2020) Illuminating dark fishing fleets in North Korea. *Science Advance* **6**, eabb1197.

Rosa A, Yamamoto J and Sakurai Y (2011) Effects of environmental variability on the spawning areas, catch, and recruitment of the Japanese common squid, *Todarodes pacificus* (Cephalopoda: Ommastrephidae), from the 1970s to the 2000s. *ICES Journal of Marine Science* **68**, 1114–1121.

Sakurai Y, Kidokoro H, Yamashita N, Yamamoto J, Uchikawa K and Takahara H (2013) *Todarodes pacificus*, Japanese common squid. In Rosa R, Pierce G and O'Dor R (eds), *Advances in Squid Biology, Ecology and Fisheries*, 2nd Edn. New Yolk: Nova Science Publishers, Inc., pp. 249–271.

Sakurai Y, Kiyofuji K, Saitoh S, Goto T and Hiyama Y (2000) Changes in inferred spawning areas of *Todarodes pacificus* (Cephalopoda: Ommastrephidae) due to changing environmental conditions. *ICES Journal of Marine Science* **57**, 24–30.

Schöne BR, Zhang Z, Radermacher P, Thébault J, Jacob DE, Nunn EV and Maurer A-F (2011) Sr/Ca and Mg/Ca ratios of ontogenetically old, long-lived bivalve shells (*Arctica islandica*) and their function as paleotem perature proxies. *Palaeogeography, Palaeoclimatology, Palaeoecology* **302**, 52–64.

Shibano R, Morimotoa A, Takayama K, Takikawa T and Ito M (2019) Response of lower trophic ecosystem in the Japan Sea to horizontal nutrient flux change through the Tsushima Strait. *Estuarine, Coastal and Shelf Science* **229**, 106386.

Shirai K, Schöne BR, Miyaji T, Radarmacher P, Krause RA and Tanabe K (2014) Assessment of the mechanism of elemental incorporation into bivalve shells (*Arctica islandica*) based on elemental distribution at the microstructural scale. *Geochimica et Cosmochimica Acta* **126**, 307–320.

Takahara H, Kidokoro H and Sakurai Y (2017) High temperatures may halve the lifespan of the Japanese flying squid, *Todarodes pacificus*. *Journal of Natural History* **51**, 2607–2614.

Vander Putten E, Dehairs F, Keppens E and Baeyens W (2000) High resolution distribution of trace elements in the calcite shell layer of modern *Mytilus edulis*: environmental and biological controls. *Geochimica et Cosmochimica Acta* **64**, 997–1011.

Wanamaker ADJ, Kreutz KJ, Wilson T, Borns HWJ, Introne DS and Feindel S (2008) Experimentally determined Mg/Ca and Sr/Ca ratios in juvenile bivalve calcite for *Mytilus edulis*: implications for paleotemperature reconstructions. *Geo-Marine Letters* **28**, 359–368.

Yamaguchi T, Aketagawa T, Miyamoto M, Hirose N and Matsuyama M (2018) The use of statolith analyses and particle-tracking experiments to reveal the migratory routes of the swordtip squid (*Uroteuthis edulis*) caught on the Pacific side of Japan. *Fisheries Oceanography* **27**, 517–524.

Yamaguchi T, Kawakami Y and Matsuyama M (2015) Migratory routes of the swordtip squid *Uroteuthis edulis* inferred from statolith analysis. *Aquatic Biology* **24**, 53–60.