

Ontogenetic difference of beak elemental concentration and its possible application in migration reconstruction for *Ommastrephes bartramii* in the North Pacific Ocean

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Abstract

The migration route of oceanic squid provides critical information for us to understand their spatial and temporal variations. Mark-recapture and electronic tags tend to be problematic during processing. Cephalopod hard structures such as the beak, containing abundant ecological information with stable morphology and statolith-like sequences of growth increments, may provide information for studying spatio-temporal distribution. In this study, we developed a method, which is based on elemental concentration of beaks at different ontogenetic stages and sampling locations, to reconstruct the squid migration route. We applied this method to *Ommastrephes bartramii* in the North Pacific Ocean. Nine trace elements were detected in the rostrum sagittal sections (RSS) of the beak using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). For those elements, significant differences were found between the different ontogenetic stages for phosphorus (P), copper (Cu) and zinc (Zn). Sodium (Na), P and Zn were chosen as indicators of sea surface temperature (SST) and a regression model was estimated. The high probability of occurrence in a particular area represented the possible optimal squid location based on a Bayesian model. A reconstructed migration route in this study, combining all the locations at different ontogenetic stages, was consistent with that hypothesized in previous studies. This study demonstrates that the beak can provide useful information for identifying the migration routes of oceanic squid.

Key words: *Ommastrephes bartramii*, beak, trace element, ontogenetic stage, migration route

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1 Introduction

The cephalopod, an important oceanic invertebrate species, plays an important role at the median trophic level in the marine ecosystem (Navarro et al., 2013). Oceanic species, especially Ommastrephids, support a high proportion of the world's cephalopod fisheries, with catches from 2.0 million metric tons in the 1990s to a peak of 3.5 million metric tons in 2014 (<http://www.fao.org/fishery/statistics>).

In the North Pacific Ocean, a larger Ommastrephid species, *Ommastrephes bartramii*, is widely distributed and commercially exploited by the East Asian countries (Bower and Ichii, 2005; Chen et al., 2008; Jereb and Roper, 2010). Similar to other oceanic species, *O. bartramii* migrates a considerable distance over its ontogenetic stages (Ichii et al., 2009). The most popular

methods to investigate cephalopod migration are external tag mark-recapture and electronic tags (Wearmouth et al., 2013; Mereu et al., 2015). However, jigging fishing activity cannot cover the entire spatial range of the squid feeding grounds, and the high rate of arm/tentacle breakage during jigging leads to a low recapture rate, making the external tag an unsuitable approach for squid study. Electronic tags in the squid's mantle or fin are not easy to monitor as the tagged individuals are usually out of the range of recording devices (Semmens et al., 2007).

Cephalopods always seek a suitable habitat to maintain their daily activity to achieve an optimum oceanographic environment at all life stages, and consume prey to grow as fast as possible (Boyle and Rodhouse, 2005). Thus their surrounding environment and food availability are the two main drivers for squid

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movement and migration (Ichii et al., 2004; Watanabe et al., 2008). There is no doubt that oceanic squid are exposed to different environments at different life stages (Murata and Nakamura, 1998; Ichii et al., 2004). Female *O. bartramii* prefer to spawn in areas with an SST range from 21°C to 25°C, which is the optimum temperature for egg production (Vijai et al., 2015). After hatching, the paralarvae remain near the spawning ground with the current for the first 90 d at a preferred temperature of 20°C to 22°C (Nishikawa et al., 2014). The squid begin to prey on food when they have the ability of independent movement. It has also been reported that juvenile squid always inhabit food-rich areas with SST > 21°C (Kato et al., 2014). Adult squid grow fast with a voracious feeding behavior, so they tend to gather in high productivity areas, which are those feeding grounds with SST 16–19°C, as has been suggested by most researchers in previous studies (Tian et al., 2009; Alabia et al., 2015). Temperature is an indicator that directly influences squid growth and their food distribution, and also indirectly influences squid migration.

Understanding the relationship between squid movement behavior and their key environment is thus a priority to study the possible migration routes. The statolith is often referred to as a “black box” because it records the ecological information of the whole life stages of the squid (Arkhipkin, 2005). The age and growth pattern can be analyzed through the increments in the statolith microstructure (Arkhipkin and Shcherbich, 2012), and the distinctive chemical composition also reveals the ambient experienced by the squid at different life stages (Liu et al., 2015a; Arbuckle and Wormuth, 2014). The Sr/Ca ratio is an important sea surface temperature (SST) indicator for investigating the migration strategies of different ommastrephid squid (Ikeda et al., 1997, 2002, 2003).

As an important hard structure in squid, the beak, which has a larger size than the statolith and a unique morphology, has become an alternative tool for investigating squid biology in recent years (Chen et al., 2012; Franco-Santos and Vidal, 2014; Liu et al., 2015b; Fang et al., 2016a). A sequence of growth increments were also observed in the microstructure in the rostrum sagittal sections of the beak, which suggests that the beak can be used in the study of age and growth for squid (Liu et al., 2015c; Fang et al., 2016b). Stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in the squid beak also reflect the variation of trophic position and foraging areas at intra- and inter-species levels (Jennings and Cogan, 2015; Fang et al., 2016b). The beak is a compound that consists of protein and polysaccharide (chitin), and the pigmented part, which contains high-density catecholic-histidine cross linking, has also attracted the attention of researchers who have tried to apply it in new biomaterials (Miserez et al., 2007, 2008, 2010). Some studies have tried to explore the inorganic elements in the cephalopod beak, although this is not a common material for biochemical analysis. Rodríguez-Navarro et al. (2006) suggested that the trace element profiles (P and Se) in the beak could indicate a gradual descent to deeper waters during the life of the giant squid (*Architeuthis* sp.). Thus, analyzing the trace elements in the beak is a potential method to investigate the pattern of squid movements.

In this study, we quantitatively measured the main trace elements to estimate the category and concentration of the *O. bartramii* beak and compared the possible elemental difference at different life stages and hatching seasons. The migration route of *O. bartramii* was also reconstructed based on the relationship between sea surface temperature and selected beak elemental signatures. This study shows the potential of using microchemistry to improve our understanding of the migration pattern of *O. bartramii* in the Northwest Pacific Ocean.

2 Materials and methods

2.1 Sample collection and measurements

A total of 62 squid samples were randomly obtained from the commercial oceanic jigging vessel F/V *Jinhai 827* which operated in the Northwest Pacific Ocean (39°–44°N, 152°–157°E) from July to November in 2010 and 2011. Samples were immediately frozen at –18°C for future laboratory work.

Dorsal mantle length (ML) was measured to the nearest 1 mm after thawing in the laboratory. The upper and lower beaks were dissected from the buccal mass, washed with fresh water and stored in 75% ethyl alcohol. As the upper beak is larger than the lower beak, and age estimation has been tested on the rostrum of the upper beak in previous studies (Liu et al., 2015c; Fang et al., 2016b), we used the upper beak for the next step.

2.2 Age estimation and back-calculation

The statolith is often used for cephalopod age estimation (Arkhipkin and Shcherbich, 2012), but the tiny size of a statolith and the cumbersome process protocol complicates the interpretation of the ageing results (Arkhipkin, 2012). Thus the utilization of other hard structures has been proposed (Moltschaniwskyj and Cappel, 2009). Liu et al. (2015c) suggested that the increments in the beak rostrum had a deposition pattern similar to a statolith increment, and Fang et al. (2016b) suggested that the beak could be a useful material for age estimation for *O. bartramii*. We used 40 upper beak rostrum sagittal sections (RSS) for ageing and back-calculating the hatching dates based on estimated age and date of capture. All the increments in the statolith sample were read by two researchers, and the result is the average age with standard deviation.

2.3 Sea surface temperature (SST) data

Sea surface temperature (SST) is considered to be an important factor affecting the incremental deposition in the statolith, as the squid take up trace elements at night on the sea surface (Bettencourt and Guerra, 2000). Perales-Raya et al. (2014a) reported that a high variation of SST coincided with the locations of the marks on the beak of *Octopus vulgaris*, and that beak growth was closely related to the environment, including SST (Perales-Raya et al., 2014b). Thus the SST has a high correlation with beak growth. Real-time SST data were recorded with a highly accurate conductivity and temperature recorder (SBE 37-SM MicroCAT; Sea-Bird Electronics, Bellevue, WA) at each sampling station. In order to get precise temperatures, the weekly SST in the Northwest Pacific Ocean during 2009 to 2011 was downloaded from the National Oceanic and Atmospheric Administration website (<http://oceanwatch.pifsc.noaa.gov/>) with spatial resolution of 0.1°×0.1°. The SST of the week to the nearest time when a back-calculated ontogenetic stage occurred was used as the SST for the life history stage.

2.4 Trace element analysis

All the 40 beak samples were selected for trace element analysis (Table 1). After reading the increments from the RSS of the beak, the polished RSS was washed in ultra-pure water for 5 min, and then rinsed at least twice before being air dried. Laser ablation was used to sample in different areas of the beak, the location representing each different ontogenetic stage as referred to by Young and Harman (1988) and Liu et al. (2015c). The growth of the beak starts from the tip (Hernández-López et al., 2001; Perales-Raya et al., 2010), so the tip (0–5 increments) of the RSS represents the embryonic stage, the second spot, located in ap-

Table 1. Sample information

ID	Capture time (year/month/day)	East longitude	North latitude	Body weight/g	Mantle length/mm	Sex	Sexual maturity stage	Age/d	Hatching time
1881	2010/07/13	153°36′	39°24′	322.9	234	♀	II	162	2010/01/31
1917	2010/07/13	153°36′	39°24′	305	221	♀	I	142	2010/02/20
1922	2010/07/13	153°36′	39°24′	425.2	243	♂	II	176	2010/01/18
1931	2010/07/13	153°36′	39°24′	244.3	213	♂	I	129	2010/03/06
532	2010/08/16	152°14′	40°11′	275.3	220	♂	I	171	2010/02/26
544	2010/08/16	152°14′	40°11′	311	216	♀	I	158	2010/03/11
419	2010/08/29	152°49′	42°21′	298.7	227	♂	I	152	2010/03/30
425	2010/08/29	152°49′	42°21′	374.7	238	♀	II	169	2010/03/13
442	2010/08/29	152°49′	42°21′	364.1	242	♂	II	174	2010/03/07
444	2010/08/29	152°49′	42°21′	370.5	243	♂	I	176	2010/03/06
776	2010/09/18	154°23′	41°32′	356.3	233	♀	II	165	2010/04/06
781	2010/09/18	154°23′	41°32′	320.5	215	♀	II	154	2010/04/17
1693	2010/10/12	156°47′	42°35′	608	282	♂	II	229	2010/02/25
1872	2010/11/05	154°07′	41°57′	448.1	249	♂	III	185	2010/05/04
26	2010/07/19	157°52′	40°44′	608	214	♂	I	200	2009/12/31
32	2010/07/19	157°52′	40°44′	448.1	225	♂	I	185	2010/01/15
39	2010/07/19	157°52′	40°44′	608	224	♂	I	229	2009/12/02
137	2011/08/07	154°28′	42°25′	1 024.8	326	♀	II	281	2010/10/30
190	2011/08/09	153°58′	42°27′	226.1	203	♂	I	112	2011/04/19
203	2011/08/20	152°58′	42°05′	805.2	289	♀	II	265	2010/11/28
211	2011/08/20	152°58′	42°05′	302.2	239	♂	I	154	2011/03/19
212	2011/08/20	152°58′	42°05′	759.6	275	♀	III	254	2010/12/09
66	2011/09/03	150°59′	42°58′	1 030.5	327	♀	II	282	2010/11/25
268	2011/09/11	154°32′	42°50′	916.8	302	♀	IV	254	2010/12/31
272	2011/09/11	154°32′	42°50′	393	247	♀	II	182	2011/03/13
279	2011/09/11	154°32′	42°50′	453.8	251	♀	II	188	2011/03/07
296	2011/09/11	154°32′	42°50′	488	269	♂	II	212	2011/02/10
334	2011/09/13	152°30′	43°21′	357.9	238	♂	I	169	2011/03/28
348	2011/09/13	152°30′	43°21′	363.3	243	♂	I	176	2011/03/21
352	2011/09/13	152°30′	43°21′	397.4	251	♂	II	188	2011/03/09
306	2011/09/28	154°42′	42°59′	539	271	♂	III	215	2011/02/25
307	2011/09/28	154°42′	42°59′	790.5	301	♂	III	252	2011/01/18
313	2011/09/28	154°42′	42°59′	391.2	245	♀	II	179	2011/04/02
228	2011/10/09	156°08′	43°13′	340.2	222	♀	I	144	2011/05/18
237	2011/10/09	156°08′	43°13′	390	238	♀	I	169	2011/04/23
665	2011/10/16	151°45′	42°14′	879.5	298	♀	III	234	2011/02/24
672	2011/10/16	151°45′	42°14′	896.1	297	♀	II	227	2011/03/03
106	2011/10/23	150°21′	41°21′	934.8	310	♀	II	263	2011/02/02
115	2011/11/08	150°49′	40°58′	2 027	406	♀	IV	359	2010/11/13
116	2011/11/08	150°49′	40°58′	1 037	320	♀	III	274	2011/02/06

proximately the 30th increment represents the paralarval stage, the third spot located around the 60th increment represents the juvenile stage, the fourth spot located at about the 120th increment represents the sub-adult stage, and the intersection point between the hood and crest represents the sub-adult stage, and also indicates the capture time (Fig. 1).

Compared to the statolith, the main elements in a cephalopod beak are ^{43}Ca , ^{23}Na , ^{24}Mg , ^{31}P , ^{39}K , ^{55}Mn , ^{63}Cu , ^{66}Zn , ^{83}Se , and ^{88}Sr . Therefore, an analysis of the above ten elemental signatures was performed using a 193-nm frequency-quadrupled ArF excimer laser (GeoLas, 2005; Lambda Physik, Göttingen, Germany) with pulse of 8 Hz, energy of 6.0 J/cm², and sampling spot diameter of 44 μm . This laser ablation station was connected to an Agilent 7500a inductively coupled plasma mass spectrometer (ICP-MS) with sample gas Helium of 0.9 L/min and flow gas of Argon 0.92 L/min (Agilent Technologies, Santa Clara, CA). Each

analysis incorporates 20 s of background acquisition followed by 60 s of sample ablation to the corrected sample concentrations

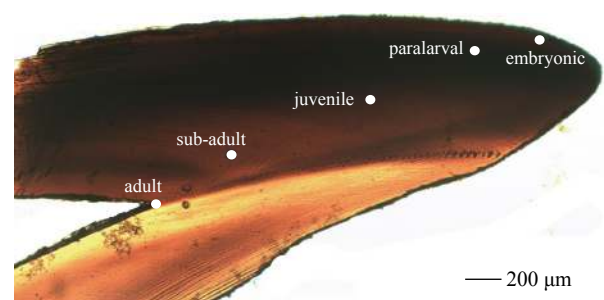


Fig. 1. Detected spots of beak microstructure representing different ontogenetic stages by LA-ICP-MS.

(Elsdon and Gillanders, 2005; Liu et al., 2016). An ANOVA test and a *t*-test were conducted to evaluate the possible difference between the different ontogenetic stages and seasonal hatching groups, respectively.

2.5 Hypothesis and assumptions

Each of the ten elements (^{43}Ca , ^{23}Na , ^{24}Mg , ^{31}P , ^{39}K , ^{55}Mn , ^{63}Cu , ^{66}Zn , ^{79}Se , ^{88}Sr) on the tip of the RSS (spot 5) was analyzed, and stepwise multiple linear regression analyses were used to predict the optimal SST. The best fit model was selected by the stepwise removal of the elements based on Akaike information criterion (AIC) (Akaike, 1974). It is possible to estimate the optimal SST for the earlier ontogenetic stage on the basis of the relationship between SST and the relevant element. The possible spatial locations corresponding to the suitable SST can be determined according to the elemental concentration (Liu et al., 2016). As we know the elements concentration of squid beak in different life time, it is easily to find the suitable SST according to the established relationship between SST and relevant element. Considering the squid swimming ability, the possible location (longitude and latitude) of SST will also revealed in the SST database mentioned in above content using Bayesian model.

All the samples were taken in a similar area and shared the same habitat. We identified that they belonged to the same cohort according to previous studies (Fang et al., 2016b). Therefore the squid are likely to experience a similar environment during their life history, even if they were not born at same time. Finally, the deduced migration routes were connected by combining the

optimal locations (highest occurrence area in each stage) at all five ontogenetic stages.

The timing of each ontogenetic stage was estimated following Liu et al. (2016). The squid were assumed to swim in a range of defined suitable SST values, and the maximum swimming speed of *O. bartramii*, 20 km per day (Tanaka, 2001), was used for calculating the possible migration range. The possibility of a squid occurrence area was also calculated following Liu et al. (2016). The best model selections and migration reconstructions were all carried out with R 3.1.3 (R Core Team, 2015) using the package “MASS” (Venables and Ripley, 2002) and “geoR” (Ribeiro and Diggle, 2001), respectively.

3 Results

3.1 Element concentration in the beak

The highest concentration of the analyzed elements was Ca, with an average $\pm$ standard deviation $(1\,497.05 \pm 628.8) \times 10^{-6}$ (Table 2). Magnesium (Mg) had the second highest concentration with $(597.21 \pm 279.34) \times 10^{-6}$ (Table 2). The other elements with concentrations higher than 100×10^{-6} were phosphorus (P), sodium (Na) and potassium (K) (Table 2). Strontium (Sr), of which there are high concentrations in the statolith, had the lowest concentration in the beak (Table 2). Concentrations of all the elements were unstable with a relatively high standard deviation in the results (Table 2). The concentration of Se in the RSS was too low to be measured.

Table 2. Element concentration and element/Ca ratios of *O. bartramii* beak measured by LA-ICP-MS

Element	Concentration/ 10^{-6}		Element/Ca ratios/ $\mu\text{mol}\cdot\text{mol}^{-1}$	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Ca	436.92–3 134.21	1 497.05 $\pm$ 628.80	–	–
Mg	180.52–2 967.55	597.21 $\pm$ 279.34	138.95–2 767.17	461.74 $\pm$ 203.91
P	200.07–1 885.68	519.41 $\pm$ 260.56	70.41–1 824.11	596.00 $\pm$ 326.69
Na	151.04–1 617.58	309.39 $\pm$ 162.59	161.61–1 193.10	264.02 $\pm$ 82.42
K	160.86–775.56	244.39 $\pm$ 164.62	120.19–616.88	217.84 $\pm$ 83.40
Cu	30.40–312.65	81.21 $\pm$ 41.68	10.09–365.00	61.15 $\pm$ 35.10
Zn	10.29–106.92	42.40 $\pm$ 26.09	10.37–134.70	36.48 $\pm$ 17.86
Sr	1.10–32.01	10.12 $\pm$ 6.70	1.28–25.79	7.00 $\pm$ 4.41

3.2 Elemental signature difference among ontogenetic stages and hatching times

The elemental signatures at the embryonic and paralarval stage were lower than at the other ontogenetic stages, as the elements accumulated with squid growth. Mg/Ca mostly remained at the same level until the adult stage (Fig. 2a). The concentrations of Na/Ca and K/Ca increased during the juvenile and sub-adult stage, but decreased at the adult stage (Fig. 2a). Concentrations of P/Ca and Zn/Ca increased drastically from the paralarval to the juvenile stage, and then fluctuated between the juvenile and adult stage (Figs 2a and b). The concentration of Cu/Ca was highest in the juvenile stage, and then decreased (Fig. 2b). The ANOVA test showed that P/Ca, Cu/Ca and Zn/Ca had a significant difference at different ontogenetic stages ($P < 0.05$), and the embryonic and paralarval stage had a significant difference when tested post-hoc.

All of the squid sampled in this study were hatched between May and October of the previous year. Thus we separated the samples into two seasonal groups, the winter hatching group (hatching time during October to February) and spring hatching group (hatching time during March to May). Statistical analysis

showed that none of the elements differed between the two seasonal groups at every ontogenetic stage ($P > 0.05$), although the concentration of P/Ca fluctuated in different patterns during the paralarval and adult stages (Fig. 3a), and the Cu/Ca showed a reverse pattern during the sub-adult and adult stages (Fig. 3b).

3.3 Migration route reconstruction

As the stepwise regression analysis describes (Table 3), sodium, phosphorus and zinc are suitable indicators of SST. The derived regression model can be described as follows: $\text{SST} = 20.367 - 0.0076[\text{Na}] - 0.0009[\text{P}] + 0.0201[\text{Zn}]$ ($R^2 = 0.552$, $P < 0.01$).

The ages selected from the five analyzed locations on the RSS of the beak represented the five ontogenetic stages, with an age of 0 days for an embryo, 27 (18–35) d for paralarvae, 58 (45–70) d for juveniles, 124 (90–139) d for sub-adults, and 162 (118–180) d for adults (Table 4). The value range of Na/Ca, P/Ca and Zn/Ca is shown in Table 5. In the embryonic and paralarval stages, the value of Na/Ca is nearly unchanged although P/Ca and Zn/Ca increased steadily. The value of the three selected elements performed a “U-shape” tendency during the juvenile to adult stages. (Table 5).

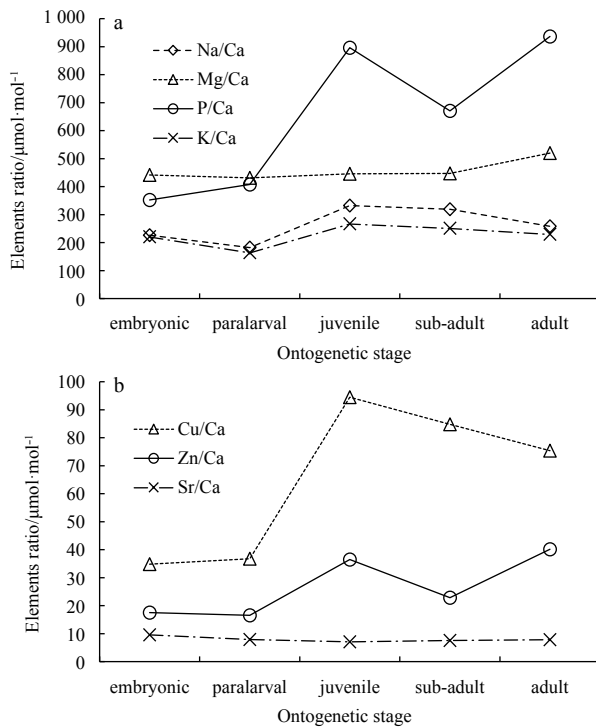


Fig. 2. Element/Ca ratios of *O. bartramii* beak at different ontogenetic stages.

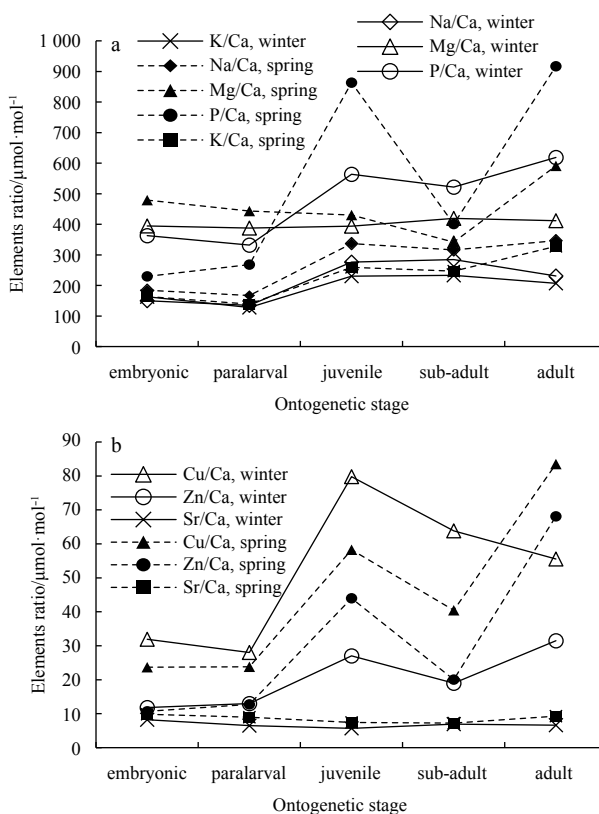


Fig. 3. Element/Ca ratios of *O. bartramii* beak at different hatching seasons.

The occurrence probability of squid at different ontogenetic stages was calculated and the results are shown in Fig. 4. The mi-

gration route can be described by connecting the highest occurrence probability during the consecutive ontogenetic stages (Fig. 5). A south-north migration trace for *O. bartramii* is shown in Fig. 5.

4 Discussion

The squid movement pattern is closely related to the ambient environment (Sim et al., 2001). The eggs and paralarvae passively drift with the current, and then the squid actively migrate in a relatively long journey in line with body growth (O'Dor and Balch, 1985). Feeding and spawning are the two reasons that cause the squid to migrate (Semmens et al., 2007). Thus a good understanding of migration routes can enhance our knowledge of squid population dynamics and spatial distribution leading to improved assessment and management of this important fisheries resource.

The composition of trace elements in the beak was quite different from those in the statolith (Table 2). Most of the metal elements, including magnesium, sodium, potassium, copper and zinc, had already been detected in the statolith, and some of the elements were taken up from the water mass and then deposited in the statolith (Liu et al., 2013; Arbuckle and Wormuth, 2014). Strontium is the second highest element in the statolith, but in this study the value of strontium in the beak was low (only about 10×10^{-6}) (Table 2). Phosphorus is a remarkable trace element in the beak, which is rarely seen in the statolith (Table 2). Unlike the calcareous tissues of the statolith, the beak is a fully organic hard structure in which any mineral phases and metals are mostly absent (Miserez et al., 2007). Thus the beak would experience a series of protein syntheses to prompt cell proliferation and growth, and phosphorus plays an important role connecting different kinds of amino acid and promotes protein synthesis (Van-Bogelen et al., 1996). It is, therefore, easy to understand the reason for the high concentration of phosphorus in a squid beak. Selenium was not measured in this study. As the species and the environment become more varied, more element concentrations in the beak should be tested in the future.

Phosphorus, copper and zinc obviously fluctuated highly at the different ontogenetic stages, although only copper showed significant differences (Fig. 2b). These three elements increased greatly from the paralarval to the juvenile stage (Fig. 2). With squid growth, the transformation of feeding behavior of paralarvae to juveniles moves from a passive uptake of zooplankton to gradually active preying on small crustaceans and fish (Watanabe et al., 2008), and phosphorus increases to promote protein synthesis, allowing beak cells to grow faster to adapt to this behavior change. The hard and colored part of the beak enables the squid to eat large prey with hard skins. This beak pigmentation process is a kind of melanogenesis, and copper and zinc are two important trace elements for the speed of melanin biosynthesis (Swan, 1974; Slominski et al., 2004). The squid would then migrate from the hatching area to the feeding ground during the juvenile to sub-adult stage. In this phase, the food composition does not change too much but most of the nutrition is supplied for migration movement, so the concentration of the above three elements decreases, but is still higher than that at the paralarval stage (Fig. 2). Squid need enough food for gametogenesis during the sub-adult to adult stage. Therefore, the beak experiences a second round of growth and becomes stiffer to allow the squid to prey on larger fish and other squid (Watanabe et al., 2004; Fang et al., 2016c), so phosphorus and zinc increase again to a high level (Fig. 2). This may explain the "U-shape" tendency of P/Ca and Zn/Ca during the juvenile to adult stages. A decrease in copper may be caused by an antagonistic effect with zinc in an organism

Table 3. Results of stepwise regression analysis of different elements

Step	Removed	d.f.	Std	Residual d.f.	Residual std	AIC
1	–	–	–	22	105.227	53.648
2	Cu	1	0.140 434	23	105.369	51.688
3	K	1	3.075 477	24	108.444	50.551
4	Mg	1	2.717 551	25	111.162	49.294
5	Sr	1	6.416 297	26	117.578	48.977

Variable	Coefficient	St error	T value	P value	95% confident interval	
					Upper	Lower
Intercept	20.367	0.655	31.095	<0.001	19.021	21.714
Na slope	–0.007 6	0.002 1	–3.493	0.001 7	–0.012	–0.003
P slope	–0.000 9	0.000 5	–1.784	0.086 1	–0.002	0.000 1
Zn slope	0.020 1	0.006 1	3.282	0.002 9	0.007	0.032 8

Table 4. Age and date at each ontogenetic stage in squid sample

ID	Age					Date (year/month/day)				
	Embryo	Paralarvae	Juvenile	Sub-adult	Adult	Embryo	Paralarvae	Juvenile	Sub-adult	Adult
1922	0	23	48	110	144	2010/01/18	2010/02/10	2010/03/07	2010/05/08	2010/06/11
1881	0	24	54	114	161	2010/01/31	2010/02/24	2010/03/26	2010/05/25	2010/07/11
1917	0	21	52	116	157	2010/02/20	2010/03/13	2010/04/13	2010/06/16	2010/07/27
1693	0	27	56	127	171	2010/02/24	2010/03/23	2010/04/21	2010/07/01	2010/08/14
137	0	25	57	112	162	2010/10/30	2010/11/24	2010/12/26	2011/02/19	2011/04/10
203	0	35	69	138	180	2010/11/28	2011/01/02	2011/02/05	2011/04/15	2011/05/27
211	0	26	55	120	154	2011/03/19	2011/04/14	2011/05/13	2011/07/17	2011/08/20
212	0	31	62	135	176	2010/12/09	2011/01/09	2011/02/09	2011/04/23	2011/06/03
66	0	32	65	121	168	2010/11/25	2010/12/27	2011/01/29	2011/03/26	2011/05/12
115	0	27	60	139	180	2010/12/20	2011/01/16	2011/02/18	2011/05/08	2011/06/18
268	0	29	53	125	162	2010/12/31	2011/01/29	2011/02/22	2011/05/05	2011/06/11
307	0	28	69	126	172	2011/01/18	2011/02/15	2011/03/28	2011/05/24	2011/07/09
532	0	26	56	122	171	2010/02/26	2010/03/24	2010/04/23	2010/06/28	2010/08/16
544	0	33	52	128	158	2010/03/11	2010/04/13	2010/05/02	2010/07/17	2010/08/16
106	0	31	59	135	159	2011/02/02	2011/03/05	2011/04/02	2011/06/17	2011/07/11
116	0	32	70	132	160	2011/02/06	2011/03/10	2011/04/17	2011/06/18	2011/07/16
296	0	26	64	129	170	2011/02/10	2011/03/08	2011/04/15	2011/06/19	2011/07/30
26	0	29	62	128	168	2009/12/31	2010/01/29	2010/03/03	2010/05/08	2010/06/17
32	0	21	54	120	178	2010/01/15	2010/02/05	2010/03/10	2010/05/15	2010/07/12
39	0	26	64	126	162	2009/12/02	2009/12/28	2010/02/04	2010/04/07	2010/05/13
306	0	30	66	135	168	2011/02/25	2011/03/27	2011/05/02	2011/07/10	2011/08/12
1931	0	19	45	90	118	2010/03/06	2010/03/25	2010/04/20	2010/06/04	2010/07/02
444	0	24	62	126	156	2010/03/06	2010/03/30	2010/05/07	2010/07/10	2010/08/09
776	0	32	57	123	165	2010/04/06	2010/05/08	2010/06/02	2010/08/07	2010/09/18
781	0	18	49	115	154	2010/04/17	2010/05/05	2010/06/05	2010/08/10	2010/09/18
442	0	28	52	130	158	2010/03/07	2010/04/04	2010/04/28	2010/07/15	2010/08/12
419	0	27	45	112	142	2010/03/30	2010/04/26	2010/05/14	2010/07/20	2010/08/19
352	0	32	60	126	167	2011/03/09	2011/04/10	2011/05/08	2011/07/13	2011/08/23
279	0	31	61	129	171	2011/03/07	2011/04/07	2011/05/07	2011/07/14	2011/08/25
665	0	25	49	134	169	2011/02/24	2011/03/21	2011/04/14	2011/07/08	2011/08/12
672	0	28	54	111	149	2011/03/03	2011/03/31	2011/04/26	2011/06/22	2011/07/30
334	0	32	65	125	153	2011/03/28	2011/04/29	2011/06/01	2011/07/31	2011/08/28
313	0	19	58	132	164	2011/04/02	2011/04/21	2011/05/30	2011/08/12	2011/09/13
237	0	29	57	128	158	2011/04/23	2011/05/22	2011/06/19	2011/08/29	2011/09/28

(Fig. 2).

It is possible to predict squid migration routes and timing with the development of new methodologies and devices. SST is an important environmental factor that limits the distribution of *O. bartramii*. Optimum SST controls the movement of squid, which is why specific locations and temperature for *O. bartramii* at different life stages have been investigated in previous studies

(Table 6). Some suitable elemental signatures were selected as temperature indicators to predict areas of high probability of occurrence for different ontogenetic stages of *O. bartramii* in the North Pacific Ocean. The range of highest probability occurrence between embryonic and paralarval stages was similar (Fig. 4). It is well-known that squid at the early life stage (e.g., egg, paralarvae, even juvenile) lack the ability to actively swim and only pass-

Table 5. Concentration ratio of Na/Ca, P/Ca and Zn/Ca at different life stages of each sample

ID	Embryo			Paralarvae			Juvenile			Sub-adult			Adult		
	Na/Ca	P/Ca	Zn/Ca	Na/Ca	P/Ca	Zn/Ca	Na/Ca	P/Ca	Zn/Ca	Na/Ca	P/Ca	Zn/Ca	Na/Ca	P/Ca	Zn/Ca
1922	204.61	302.95	19.22	150.05	621.57	17.33	245.57	809.09	25.51	111.82	623.94	13.52	277.48	697.04	11.19
1881	129.97	361.23	20.63	212.93	411.54	22.80	375.16	572.23	18.65	353.80	383.07	7.11	86.92	491.61	44.60
1917	106.37	60.48	4.32	94.76	278.93	55.34	244.49	366.96	99.63	254.99	585.97	118.26	62.58	400.71	2.92
1693	170.51	266.08	8.62	169.10	322.97	12.25	139.53	674.95	8.44	242.42	420.02	29.23	116.53	444.84	47.64
137	123.99	11.85	0.55	100.95	18.15	0.40	381.62	33.02	0.81	462.10	47.24	0.81	179.58	32.89	0.62
203	156.88	201.52	6.75	178.56	286.75	23.56	201.96	465.87	7.56	225.45	576.55	15.87	187.89	486.12	37.51
211	72.69	178.65	3.56	98.57	278.93	4.86	150.68	358.63	8.94	200.78	340.25	7.12	178.25	256.12	10.84
212	127.52	280.66	2.75	143.18	370.68	3.05	178.22	410.82	7.45	254.99	387.66	6.75	187.44	310.75	5.73
115	373.51	68.68	3.29	218.72	71.24	4.41	378.69	101.50	7.14	787.46	284.10	19.05	381.84	63.60	5.79
66	69.74	30.14	3.84	37.71	33.14	2.94	119.34	124.85	9.56	171.72	105.09	7.63	210.74	409.00	9.28
268	252.55	404.13	35.91	150.71	781.94	28.32	206.40	857.16	27.12	76.12	791.58	9.26	81.10	548.00	7.35
307	97.46	88.78	5.13	245.23	58.96	4.48	615.17	197.03	25.84	694.89	281.16	22.68	295.64	81.11	7.65
532	156.42	176.21	5.22	178.77	146.78	5.97	267.83	278.63	24.32	365.88	254.12	9.78	174.84	189.87	14.70
544	189.42	210.20	7.24	165.43	320.54	6.89	301.24	579.30	19.85	477.53	379.57	10.58	298.65	502.78	17.65
106	203.22	332.63	25.64	239.44	650.34	16.68	180.71	760.96	9.16	327.57	694.29	22.37	742.66	521.26	133.18
116	55.58	16.70	1.53	47.40	27.97	1.95	57.66	76.70	7.23	109.86	124.15	14.14	124.50	143.90	13.43
296	203.29	233.83	11.72	146.58	113.40	8.35	387.13	533.66	13.64	246.64	204.14	8.26	153.64	357.40	146.41
26	156.53	215.49	4.23	185.93	325.62	5.38	275.62	587.64	9.53	385.51	604.88	18.26	245.32	514.20	15.23
32	98.78	148.32	1.85	120.45	254.63	2.87	279.22	436.51	6.52	405.78	587.12	7.25	211.12	542.69	5.36
39	168.99	267.33	4.05	186.77	314.25	6.24	324.69	508.23	8.52	444.67	742.36	7.98	305.78	695.81	8.53
306	178.22	138.60	6.97	63.90	104.88	6.55	436.19	269.57	30.36	492.34	236.02	10.21	512.56	198.68	18.90
1931	146.92	231.29	4.71	136.13	336.89	5.55	358.50	768.00	16.03	48.74	407.03	3.31	188.55	583.77	39.70
444	35.46	58.16	2.25	49.31	25.56	4.66	55.56	68.30	4.90	52.24	59.32	2.29	93.15	104.06	8.61
776	189.21	289.78	14.23	199.71	315.69	15.41	283.18	544.99	26.42	387.46	785.44	18.77	242.32	682.41	16.97
781	214.87	304.88	6.78	275.67	415.68	9.66	401.56	654.33	10.52	415.64	807.46	10.11	342.87	795.23	8.46
442	256.96	100.29	3.29	172.35	155.50	1.95	336.90	291.07	3.96	432.23	155.43	4.47	189.69	179.92	1.99
419	72.29	108.37	3.52	69.92	175.57	2.93	183.48	792.44	10.19	189.81	571.17	6.06	142.46	161.89	1.21
352	87.30	267.01	13.10	37.11	351.87	6.21	108.09	701.90	60.18	44.73	316.50	7.70	96.55	544.18	6.35
279	404.35	134.04	9.08	527.15	212.99	15.95	850.41	763.14	99.14	543.68	435.08	44.74	225.31	934.35	36.60
665	81.32	107.53	3.75	123.81	195.87	2.12	277.65	652.82	7.56	365.43	472.51	5.23	213.44	187.12	2.75
672	132.65	212.33	10.59	93.27	387.54	7.98	176.49	654.87	14.23	234.14	387.46	25.12	256.98	299.54	12.37
334	233.62	93.03	14.22	157.12	91.46	10.83	125.09	137.54	16.96	406.21	363.87	28.51	230.70	394.26	9.81
313	283.76	268.18	27.64	131.46	263.56	9.57	319.09	630.41	46.90	265.19	472.87	7.77	284.93	514.78	12.46
237	395.34	62.49	9.10	155.97	59.94	7.20	609.42	180.70	6.79	763.27	119.07	4.18	630.61	360.89	11.32

ively move with the currents (Ichii et al., 2009; Nishikawa et al., 2014, 2015). So the locations in the two early life stages were in exactly the same area and a little northeastward at the paralarval stage, due to the direction of the Kuroshio Current (Fig. 4). At the juvenile stage, all the probability ranges were extended, especially to northward of 30°N (Fig. 4). Watanabe et al. (2008) described that for the winter-spring cohort of *O. bartramii* to actively prey on its food in summer, this migration orientation would be influenced by the Kuroshio and Kuroshio Extension that transports some individuals to an offshore area (eastward to 150°E) (Nishikawa et al., 2014), which is consistent with the results shown in this study (Fig. 4). The highest probability occurrence reached 40°N in the sub-adult stage, which is the nursery grounds for *O. bartramii* (Ichii et al., 2009). Finally, the adult squid were captured by fishing vessel at 40°N to 45°N (Fig. 4). The path of the migration route in this study is approximately consistent with the previously described study in that the winter-spring cohort of *O. bartramii* make a northward migration from a lower to a higher latitude in the northern hemisphere from the embryonic to the adult stage (Ichii et al., 2009).

Compared to the location of the spawning ground in the pre-

vious study (20°–25°N) (Ichii et al., 2009; Nishikawa et al., 2014; Igarashi et al., 2017), the location of the spawning ground in this study was located at a higher latitude (25°–30°N, Fig. 4). The habitat of squid is largely influenced by environmental variability at different spatial-temporal scales (Tian et al., 2013). Therefore the variations of optimal habitat were observed both in the spawning and the feeding ground (Alabia et al., 2015, 2016). This may be one of the reasons causing the above difference. Squid hatching in different months may also lead to latitudinal variation based on a particle tracking experiment (Nishikawa et al., 2014).

Biotic factor should be considered in the early life stage migration route, although some environmental factors may impact on the paralarvae/juvenile distribution (Nishikawa et al., 2014, 2015). Temperature is indeed the main factor influencing the survival of a squid embryo (Vijai et al., 2015). Some studies have also suggested that juvenile squid cannot choose their own habitat and only float with the current, having no swimming capability (O'Dor and Balch, 1985; Staaf et al., 2008). So a current that related to squid habitat should be added as an important factor for modelling. The elemental signature at the embryonic stage is less reliable than the velocity of current, which is considered to be a

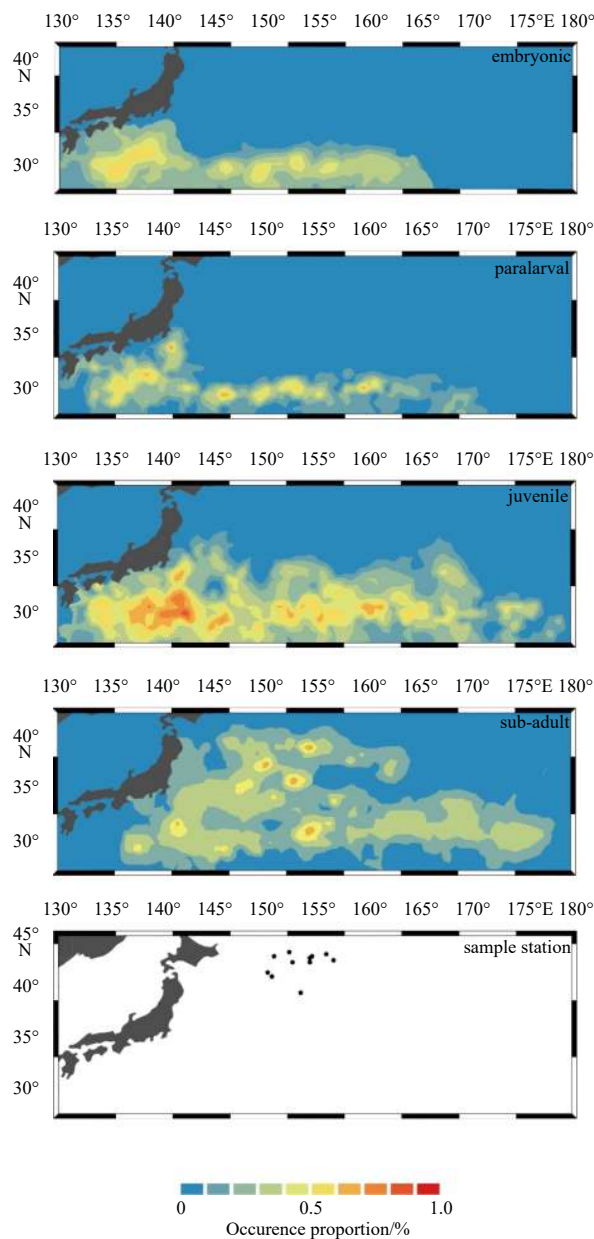


Fig. 4. Occurrence probability area of *O. bartramii* at different ontogenetic stages.

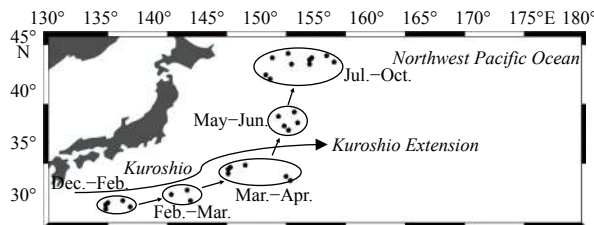


Fig. 5. Scheme of reconstructed migration route for winter-spring cohort of *O. bartramii*.

factor, due to egg hatching relying mainly on the nutrition in the yolk, which in turn depends on the predecessor (Zumholz, 2005). An individual-based biophysical-coupled model and a coupled physical-ecosystem model, which represented a more realistic methodology, were both used to simulate the possible distribu-

Table 6. Optimum sea surface temperature (SST) and latitude range for *O. bartramii* habitat at different life stages

Life stage	SST range/°C	Distribution (north latitude)	Reference
Embryo (egg)	21–25	23°–28°	Vijai et al. (2015)
Paralarvae	20–22	25°–30°	Nishikawa et al. (2014)
Juvenile	>21	28°–32°	Kato et al. (2014)
Sub-adult	16–19	35°–45°	Tian et al. (2009); Alabia et al. (2015c)
Adult	16–19	35°–45°	Tian et al. (2009); Alabia et al. (2015c)

tion and movement of the larvae of *O. bartramii* (Kato et al., 2014; Nishikawa et al., 2015).

5 Conclusions

We analyzed the category and concentration of elements in beaks of *O. bartramii*, and reconstructed the migration route based on the relationship between sea surface temperature (SST) and elemental signature. The beak mainly consists of the elements calcium, magnesium, phosphorus, sodium, potassium, copper and strontium, with the various concentrations ranking from high to low. Ontogenetic and hatching time variations were significant in the elements of phosphorus, copper and zinc. Three elements (sodium, phosphorus and zinc) were selected to fit the regression model with the corresponding SST, and the high probability occurrence range was simulated based on a Bayesian model. The reconstructed migration route was roughly consistent with previous studies, which presented a northward migration along the Kuroshio Current from the embryonic stage to the adult stage. Future study should analyze the beaks of different species of squid to compare the variation of element composition, and use more realistic methods (e.g., particle tracking simulation) to estimate the movement pattern of squid at early life stages.

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References

Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19(6): 716–723, doi: [10.1109/TAC.1974.1100705](https://doi.org/10.1109/TAC.1974.1100705)

Alabia I D, Saitoh S I, Hirawake T, et al. 2016. Elucidating the potential squid habitat responses in the central North Pacific to the recent ENSO flavors. *Hydrobiologia*, 772(1): 215–227, doi: [10.1007/s10750-016-2662-5](https://doi.org/10.1007/s10750-016-2662-5)

Alabia I D, Saitoh S I, Mugo R, et al. 2015. Seasonal potential fishing ground prediction of neon flying squid (*Ommastrephes bartramii*) in the western and central North Pacific. *Fisheries Oceanography*, 24(2): 190–203, doi: [10.1111/fog.2015.24.issue-2](https://doi.org/10.1111/fog.2015.24.issue-2)

Arbuckle N S M, Wormuth J H. 2014. Trace elemental patterns in Humboldt squid statoliths from three geographic regions. *Hydrobiologia*, 725(1): 115–123, doi: [10.1007/s10750-013-1608-4](https://doi.org/10.1007/s10750-013-1608-4)

Arkhipkin A I. 2005. Statoliths as ‘black boxes’ (life recorders) in squid. *Marine and Freshwater Research*, 56(5): 573–583, doi: [10.1071/MF04158](https://doi.org/10.1071/MF04158)

Arkhipkin A I, Shcherbich Z N. 2012. Thirty years’ progress in age determination of squid using statoliths. *Journal of the Marine Biological Association of the United Kingdom*, 92(6): 1389–1398, doi: [10.1017/S0025315411001585](https://doi.org/10.1017/S0025315411001585)

Bettencourt V, Guerra A. 2000. Growth increments and biomineralization process in cephalopod statoliths. *Journal of Experimental*

- Marine Biology and Ecology, 248(2): 191–205, doi: [10.1016/S0022-0981\(00\)00161-1](https://doi.org/10.1016/S0022-0981(00)00161-1)
- Bower J R, Ichii T. 2005. The red flying squid (*Ommastrephes bartramii*): a review of recent research and the fishery in Japan. *Fisheries Research*, 76(1): 39–55, doi: [10.1016/j.fishres.2005.05.009](https://doi.org/10.1016/j.fishres.2005.05.009)
- Boyle P, Rodhouse P G. 2005. *Cephalopods. Ecology and Fisheries*. Oxford: Blackwell Publishing, 222–233
- Chen Xinjun, Liu Bilin, Chen Yong. 2008. A review of the development of Chinese distant-water squid jigging fisheries. *Fisheries Research*, 89(3): 211–221, doi: [10.1016/j.fishres.2007.10.012](https://doi.org/10.1016/j.fishres.2007.10.012)
- Chen Xinjun, Lu Huajie, Liu Bilin, et al. 2012. Species identification of *Ommastrephes bartramii*, *Dosidicus gigas*, *Sthenoteuthis oualaniensis* and *Illex argentine* (Ommastrephidae) using beak morphological variables. *Scientia Marina*, 76(3): 473–481, doi: [10.3989/scimar.2012.76n3](https://doi.org/10.3989/scimar.2012.76n3)
- Elsdon T, Gillanders B. 2005. Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. *Marine Ecology Progress Series*, 285: 233–243, doi: [10.3354/meps285233](https://doi.org/10.3354/meps285233)
- Fang Zhou, Liu Bilin, Chen Xinjun, et al. 2016a. Sexual asynchrony in the development of beak pigmentation for the neon flying squid *Ommastrephes bartramii* in the North Pacific Ocean. *Fisheries Science*, 82(5): 737–746, doi: [10.1007/s12562-016-1011-y](https://doi.org/10.1007/s12562-016-1011-y)
- Fang Zhou, Li Jianhua, Thompson K, et al. 2016b. Age, growth, and population structure of the red flying squid (*Ommastrephes bartramii*) in the North Pacific Ocean, determined from beak microstructure. *Fishery Bulletin*, 114(1): 34–44, doi: [10.7755/FB](https://doi.org/10.7755/FB)
- Fang Zhou, Thompson K, Jin Yue, et al. 2016c. Preliminary analysis of beak stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) stock variation of neon flying squid, *Ommastrephes bartramii*, in the North Pacific Ocean. *Fisheries Research*, 177: 153–163, doi: [10.1016/j.fishres.2016.01.011](https://doi.org/10.1016/j.fishres.2016.01.011)
- Franco-Santos R M, Vidal E A G. 2014. Beak development of early squid paralarvae (Cephalopoda: Teuthoidea) may reflect an adaptation to a specialized feeding mode. *Hydrobiologia*, 725(1): 85–103, doi: [10.1007/s10750-013-1715-2](https://doi.org/10.1007/s10750-013-1715-2)
- Hernández-López J L, Castro-Hernández J L, Hernández-García V. 2001. Age determined from the daily deposition of concentric rings on common octopus (*Octopus vulgaris*) beaks. *Fishery Bulletin*, 99(4): 679–684
- Ichii T, Mahapatra K, Sakai M, et al. 2004. Differing body size between the autumn and the winter-spring cohorts of neon flying squid (*Ommastrephes bartramii*) related to the oceanographic regime in the North Pacific: a hypothesis. *Fisheries Oceanography*, 13(5): 295–309, doi: [10.1111/fog.2004.13.issue-5](https://doi.org/10.1111/fog.2004.13.issue-5)
- Ichii T, Mahapatra K, Sakai M, et al. 2009. Life history of the neon flying squid: effect of the oceanographic regime in the North Pacific Ocean. *Marine Ecology Progress Series*, 378: 1–11, doi: [10.3354/meps07873](https://doi.org/10.3354/meps07873)
- Igarashi H, Ichii T, Sakai M, et al. 2017. Possible link between interannual variation of neon flying squid (*Ommastrephes bartramii*) abundance in the North Pacific and the climate phase shift in 1998/1999. *Progress in Oceanography*, 150: 20–34, doi: [10.1016/j.pocean.2015.03.008](https://doi.org/10.1016/j.pocean.2015.03.008)
- Ikeda Y, Arai N, Kidokoro H, et al. 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, doi: [10.3354/meps251169](https://doi.org/10.3354/meps251169)
- Ikeda Y, Arai N, Sakamoto W, et al. 1997. Comparison on trace elements in squid statoliths of different species' origin: as available key for taxonomic and phylogenetic study. *International Journal of PIXE*, 7(3–4): 141–146
- Ikeda Y, Yatsu A, Arai N, et al. 2002. Concentration of statolith trace elements in the jumbo flying squid during El Niño and non-El Niño years in the eastern Pacific. *Journal of the Marine Biological Association of the UK*, 82(5): 863–866, doi: [10.1017/S0025315402006264](https://doi.org/10.1017/S0025315402006264)
- Jennings S, Cogan S M. 2015. Nitrogen and carbon stable isotope variation in northeast Atlantic fishes and squids. *Ecology*, 96(9): 2568, doi: [10.1890/15-0299.1](https://doi.org/10.1890/15-0299.1)
- Jereb P, Roper C F E. 2010. *Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. In: Myopsid and Oegopsid Squids. Vol. 2. Rome: FAO Species Catalogue for Fishery Purposes*, 269
- Kato Y, Sakai M, Mmasujima M, et al. 2014. Effects of hydrographic conditions on the transport of neon flying squid *Ommastrephes bartramii* larvae in the North Pacific Ocean. *Hidrobiológica*, 24(1): 33–38
- Liu Bilin, Cao Jie, Truesdell S B, et al. 2016. Reconstructing cephalopod migration with statolith elemental signatures: a case study using *Dosidicus gigas*. *Fisheries Science*, 82(3): 425–433, doi: [10.1007/s12562-016-0978-8](https://doi.org/10.1007/s12562-016-0978-8)
- Liu Bilin, Chen Xinjun, Chen Yong, et al. 2013. Geographic variation in statolith trace elements of the Humboldt squid, *Dosidicus gigas*, in high seas of Eastern Pacific Ocean. *Marine Biology*, 160(11): 2853–2862, doi: [10.1007/s00227-013-2276-7](https://doi.org/10.1007/s00227-013-2276-7)
- Liu Bilin, Chen Yong, Chen Xinjun. 2015a. Spatial difference in elemental signatures within early ontogenetic statolith for identifying Jumbo flying squid natal origins. *Fisheries Oceanography*, 24(4): 335–346, doi: [10.1111/fog.2015.24.issue-4](https://doi.org/10.1111/fog.2015.24.issue-4)
- Liu Bilin, Chen Xinjun, Chen Yong, et al. 2015c. Determination of squid age using upper beak rostrum sections: technique improvement and comparison with the statolith. *Marine Biology*, 162(8): 1685–1693, doi: [10.1007/s00227-015-2702-0](https://doi.org/10.1007/s00227-015-2702-0)
- Liu Bilin, Fang Zhou, Chen Xinjun, et al. 2015b. Spatial variations in beak structure to identify potentially geographic populations of *Dosidicus gigas* in the Eastern Pacific Ocean. *Fisheries Research*, 164: 185–192, doi: [10.1016/j.fishres.2014.12.001](https://doi.org/10.1016/j.fishres.2014.12.001)
- Mereu M, Agus B, Cannas R, et al. 2015. Mark-recapture investigation on *Octopus vulgaris* specimens in an area of the central western Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom*, 95(1): 131–138, doi: [10.1017/S002531541400112X](https://doi.org/10.1017/S002531541400112X)
- Miserez A, Li Youli, Waite J H, et al. 2007. Jumbo squid beaks: inspiration for design of robust organic composites. *Acta Biomaterialia*, 3(1): 139–149, doi: [10.1016/j.actbio.2006.09.004](https://doi.org/10.1016/j.actbio.2006.09.004)
- Miserez A, Schneberk T, Sun Chengjun, et al. 2008. The transition from stiff to compliant materials in squid beaks. *Science*, 319(5871): 1816–1819, doi: [10.1126/science.1154117](https://doi.org/10.1126/science.1154117)
- Miserez A, Rubin D, Waite J H. 2010. Cross-linking chemistry of squid beak. *Journal of Biological Chemistry*, 285(49): 38115–38124, doi: [10.1074/jbc.M110.161174](https://doi.org/10.1074/jbc.M110.161174)
- Moltschaniwskyj N, Cappel M. 2009. Alternatives to sectioned otoliths: the use of other structures and chemical techniques to estimate age and growth for marine vertebrates and invertebrates. In: Green B S, Mapstone B D, Carlos G, et al, eds. *Tropical Fish Otoliths: Information for Assessment, Management and Ecology*. Dordrecht: Springer, 133–173
- Murata M, Nakamura Y. 1998. Seasonal migration and diel vertical migration of the neon flying squid, *Ommastrephes bartramii*, in the North Pacific. In: Okutani T, ed. *Contributed Papers to International Symposium on Large Pelagic Squids*. Tokyo: Japan Marine Fishery Resources Research Center, 13–30
- Navarro J, Coll M, Somes C, et al. 2013. Trophic niche of squids: Insights from isotopic data in marine systems worldwide. *Deep Sea Research Part II: Topical Studies in Oceanography*, 95: 93–102, doi: [10.1016/j.dsr2.2013.01.031](https://doi.org/10.1016/j.dsr2.2013.01.031)
- Nishikawa H, Igarashi H, Ishikawa Y, et al. 2014. Impact of paralarvae and juveniles feeding environment on the neon flying squid (*Ommastrephes bartramii*) winter-spring cohort stock. *Fisheries Oceanography*, 23(4): 289–303, doi: [10.1111/fog.2014.23.issue-4](https://doi.org/10.1111/fog.2014.23.issue-4)
- Nishikawa H, Toyoda T, Masuda S, et al. 2015. Wind-induced stock variation of the neon flying squid (*Ommastrephes bartramii*) winter-spring cohort in the subtropical North Pacific Ocean. *Fisheries Oceanography*, 24(3): 229–241, doi: [10.1111/fog.12106](https://doi.org/10.1111/fog.12106)
- O'Dor R K, Balch N. 1985. Properties of *illex illecebrosus* egg masses potentially influencing larval oceanographic distribution. *NAFO Science Council Studies*, 9: 69–76

- Perales-Raya C, Almansa E, Bartolomé A, et al. 2014b. Age validation in *Octopus vulgaris* beaks across the full ontogenetic range: beaks as recorders of life events in octopuses. *Journal of Shellfish Research*, 33(2): 481–493, doi: [10.2983/035.033.0217](https://doi.org/10.2983/035.033.0217)
- Perales-Raya C, Bartolomé A, García-Santamaría M T, et al. 2010. Age estimation obtained from analysis of octopus (*Octopus vulgaris* Cuvier, 1797) beaks: Improvements and comparisons. *Fisheries Research*, 106(2): 171–176, doi: [10.1016/j.fishres.2010.05.003](https://doi.org/10.1016/j.fishres.2010.05.003)
- Perales-Raya C, Jurado-Ruzafa A, Bartolomé A, et al. 2014a. Age of spent *Octopus vulgaris* and stress mark analysis using beaks of wild individuals. *Hydrobiologia*, 725(1): 105–114, doi: [10.1007/s10750-013-1602-x](https://doi.org/10.1007/s10750-013-1602-x)
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>
- Ripley B, Ribeiro P J, Diggle P J. 2001. Spatial Statistics in R and geoR: A Package for Geostatistical Analysis. *Analysis*, 6(1): 14–15
- Rodríguez-Navarro A, Guerra A, Romanek C S, et al. 2006. Life history of the giant squid *Architeuthis* as revealed from stable isotope and trace elements signatures recorded in its beak. In: Moltschanivskij N, ed. *Cephalopod Life Cycle*. Cephalopod International Advisory Council Symposium 2006 (CIAC '06). 6–10 February 2006, Hotel Grand Chancellor, Hobart, Tasmania, 97
- Semmens J M, Pecl G T, Gillanders B M, et al. 2007. Approaches to resolving cephalopod movement and migration patterns. *Reviews in Fish Biology and Fisheries*, 17(2–3): 401–423, doi: [10.1007/s11160-007-9048-8](https://doi.org/10.1007/s11160-007-9048-8)
- Sims D W, Genner M J, Southward A J, et al. 2001. Timing of squid migration reflects North Atlantic climate variability. *Proceedings of the Royal Society B: Biological Sciences*, 268(1485): 2607–2611, doi: [10.1098/rspb.2001.1847](https://doi.org/10.1098/rspb.2001.1847)
- Slominski A, Tobin D J, Shibahara S, et al. 2004. Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiological Reviews*, 84(4): 1155–1228, doi: [10.1152/physrev.00044.2003](https://doi.org/10.1152/physrev.00044.2003)
- Staaf D J, Coop S C, Haddock S H, et al. 2008. Natural egg mass deposition by the Humboldt squid (*Dosidicus gigas*) in the Gulf of California and characteristics of hatchlings and paralarvae. *Journal of the Marine Biological Association of the UK*, 88(4): 759–770
- Swan G A. 1974. Structure, chemistry, and biosynthesis of the melanins. In: Herz W, Grisebach H, Kirby G W, eds. *Fortschritte der Chemie Organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products*. Vienna: Springer, 521–582
- Tanaka H. 2001. Tracking the neon flying squid by the biotelemetry system, in the central North Pacific Ocean. *Aquabiology (in Japanese)*, 23(6): 533–539
- Tian Siquan, Chen Xinjun, Chen Yong, et al. 2009. Evaluating habitat suitability indices derived from CPUE and fishing effort data for *Ommastrephes bartramii* in the Northwestern Pacific Ocean. *Fisheries Research*, 95(2–3): 181–188, doi: [10.1016/j.fishres.2008.08.012](https://doi.org/10.1016/j.fishres.2008.08.012)
- Tian Yongjun, Nashida K, Sakaji H. 2013. Synchrony in the abundance trend of spear squid *Loligo bleekeri* in the Japan Sea and the Pacific Ocean with special reference to the latitudinal differences in response to the climate regime shift. *ICES Journal of Marine Science*, 70(5): 968–979, doi: [10.1093/icesjms/fst015](https://doi.org/10.1093/icesjms/fst015)
- VanBogelen R A, Olson E R, Wanner B L, et al. 1996. Global analysis of proteins synthesized during phosphorus restriction in *Escherichia coli*. *Journal of Bacteriology*, 178(15): 4344–4366, doi: [10.1128/jb.178.15.4344-4366.1996](https://doi.org/10.1128/jb.178.15.4344-4366.1996)
- Venables W N, Ripley B D. 2002. *Modern Applied Statistics with S*. 4th ed. New York: Springer
- Vijai D, Sakai M, Wakabayashi T, et al. 2015. Effects of temperature on embryonic development and paralarval behavior of the neon flying squid *Ommastrephes bartramii*. *Marine Ecology Progress Series*, 529: 145–158, doi: [10.3354/meps11286](https://doi.org/10.3354/meps11286)
- Watanabe H, Kubodera T, Ichii T, et al. 2004. Feeding habits of neon flying squid *Ommastrephes bartramii* in the transitional region of the central North Pacific. *Marine Ecology Progress Series*, 266: 173–184, doi: [10.3354/meps266173](https://doi.org/10.3354/meps266173)
- Watanabe H, Kubodera T, Ichii T, et al. 2008. Diet and sexual maturation of the neon flying squid *Ommastrephes bartramii* during autumn and spring in the Kuroshio-Oyashio transition region. *Journal of the Marine Biological Association of the United Kingdom*, 88: 381–389
- Wearmouth V J, Durkin O C, Bloor I S M, et al. 2013. A method for long-term electronic tagging and tracking of juvenile and adult European common cuttlefish *Sepia officinalis*. *Journal of Experimental Marine Biology and Ecology*, 447: 149–155, doi: [10.1016/j.jembe.2013.02.023](https://doi.org/10.1016/j.jembe.2013.02.023)
- Young R E, Harman R F. 1988. “Larva”, “paralarva” and “subadult” in cephalopod terminology. *Malacologia*, 29(1): 201–207
- Zumholz K. 2005. The influence of environmental factors on the micro-chemical composition of cephalopod statoliths [dissertation]. Kiel: University of Kiel