Age-based life cycle traits of the broadclub cuttlefish *Sepia latimanus* confirmed through release–recapture experiments

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ABSTRACT: To understand the age-based life cycle traits of the broadclub cuttlefish *Sepia latimanus*, we obtained biological information about the species through release–recapture experiments, where 166 345 hatchlings with markings in their cuttlebones were released into their natural habitat of Ishigaki Island, southern Japan, and 96 adult cuttlefish were recaptured over 2 successive years. Age groups were determined based on 3 indices: the number of lamellae in the cuttlebone, dorsal mantle length (DML), and the collection date of wild cuttlefish. The length–frequency data of DML, reconstructed from 4340 cuttlebones collected from either a fish market or coastal beaches, were analysed over a period of 3 yr. The cuttlefish consisted of 2 age groups (0 yr and 1 yr), and each group is thought to die after maturation at the end of the spawning season, indicating that they are different breeding groups. The ratio of 1 yr cuttlefish to total cuttlefish was highest at the beginning of the breeding season (November) and gradually decreased throughout the breeding season. Abundance, maturation, and mating conditions of each breeding group and the estimated hatching season of cuttlefish in the wild suggest that the 0 yr and 1 yr groups contribute equally to production of offspring early in the hatching period, while the contribution of 0 yr cuttlefish is larger later in the hatching period. Offspring hatched later may return as recruits to breed as 1 yr cuttlefish the following year. Our analysis highlighted that the population of broadclub cuttlefish is composed of 2 breeding groups with alternative short and long lifespans. This is the first study in which the life cycle traits of a *Sepia* species have been successfully clarified with release–recapture experiments.

KEY WORDS: Release–recapture experiment · Cuttlefish · Cuttlebone · Age class structure · Growth · Lifespan · Mating · Maturation

INTRODUCTION

To assess and manage fishery stocks, age-based life cycle traits of target species such as recruitment, growth, and fecundity are critically important parameters for modelling population dynamics (Pierce & Guerra 1994). To estimate age-based population parameters, age determination techniques for wild animals have been developed, such as modal progression analysis of length–frequency data of fishery landings, direct age determination based on daily growth increments deposited in otoliths of fish and other bony structures, and release–recapture experiments that include release and recapture of wild-

caught or artificially incubated animals with tags or markings (Casselman 1987, Francis 1988).

In cephalopods, modal progression analyses of length–frequency data have been adopted to study their age-based growth (Voss 1983); however, recent studies suggest that length–frequency data analysis has less power to investigate age-at-size for cephalopods because of their characteristic rapid growth and the high variety in growth in a single cohort (Forsythe & Van Heukelem 1987, Jackson et al. 2000, Forsythe 2004). Recently, a direct ageing method using statoliths has been developed based on the method for fish otoliths, and reliable age-based biological data have become available for many squid species (Rodhouse & Hatfield 1990, Jackson 1994, Lipinski & Durholtz 1994). On the other hand, statolith increments are usually indistinct and irregular in cuttlefish (Natsukari & Tashiro 1991, Bettencourt & Guerra 2000, Boyle & Rodhouse 2005). Although rearing experiments have demonstrated daily deposition of statolith increments for the cuttlefish *Sepia officinalis*, the statolith rings were hardly visible in specimens older than 240 d, and thus the technique is thought to be useful only for studying juveniles (Bettencourt & Guerra 2001).

Cuttlefish are characterized by an internal shell known as the cuttlebone, which comprises a porous calcium carbonate matrix and plays an important role in controlling the buoyancy of the animal (Denton & Gilpin-Brown 1961a,b, Denton 1974, Birchall & Thomas 1983, Sherrard 2000). The cuttlebone has been used to evaluate the age and growth of cuttlefish based on the number of cuttlebone lamellae and the correlation between cuttlebone size and cuttlefish body size (Yagi 1960, Choe 1962, 1963, Almonacid-Rioseco et al. 2009). It has been documented that the lamellae are not deposited in the cuttlebone on a daily basis in some cuttlefish species (Richard 1969, Ré & Narciso 1994, Martínez et al. 2000). Thus, cuttlebone lamellae may not be used for ageing cuttlefish on a daily basis. Nevertheless, it has been suggested that the number of lamellae or lamellae thickness of the cuttlebone could be used for determining age class structure for some long-lived cuttlefish (Le Goff et al. 1998, Hall et al. 2007, Dan et al. 2011). However, to our knowledge, there have been no studies that have validated this hypothesis.

Release–recapture experiments have the advantage of obtaining biological information such as growth rate, migration, and population size during a known time at liberty. External tagging, tattooing, and branding have been used for studying cephalopod biology and ecology (Aoyama & Nguyen 1989, Naga-

sawa et al. 1993, Watanuki & Iwashita 1993, Ezzeddine-Najai 1997, Domain et al. 2000, Replinger & Wood 2007, Barry et al. 2011). To investigate the age-based life cycle traits of animals using release–recapture experiments, the age at release of hatchlings or juveniles must be known. However, external tagging, tattooing, and branding may not be suitable methods for marking small animals because these methods frequently result in the loss of tags and marks and in high mortality rates in small animals (Watanuki & Iwashita 1993, Replinger & Wood 2007). To overcome these problems, we developed a marking technique for the broadclub cuttlefish *Sepia latimanus* (Quoy & Gaimard, 1832) that stains the cuttlebone with Alizarin red S during the late embryonic developmental stage (Oka et al. 2003, Dan et al. 2008).

The broadclub cuttlefish is one of the largest species in the family Sepiidae, and can attain a dorsal mantle length of ~50 cm and weigh ~10 to 12 kg. This species is distributed throughout tropical and subtropical waters of the Indian and western Pacific Oceans (Reid et al. 2005), inhabiting waters shallower than 100 m (Sherrard 2000). In Japan, it supports local fisheries in the Nansei Islands, where it is caught with small set nets and spears (Inoha 1991). Therefore, it has been a target species for stock enhancement programmes involving the release of artificially incubated hatchlings into natural habitats (Inoha 1991, Oka et al. 2003, Dan et al. 2008). In a pilot study on stock enhancement, we developed basic techniques for broodstock management, egg collection, egg incubation management, marking of the cuttlebone, stocking of hatchlings, and investigation of recaptures (Oka et al. 1989, 2003, Oka & Tezuka 1991, 2006, Oka 1993, Dan et al. 2008). In a previous study, we released 166 345 hatchlings into their natural habitat and recaptured 96 adults over a period of 2 yr after their release (Dan et al. 2008). In addition, we developed equations to discriminate between the sexes and to reconstruct the somatic sizes from the dimensions of the cuttlebone, and suggested that the age class could be identified using the number of lamellae on the cuttlebone (Dan et al. 2011). These results have allowed further investigation of the growth and age class structure of broadclub cuttlefish populations using cuttlebones, which are usually discarded at fish markets or washed ashore on coastal beaches.

The present study is the first known attempt to apply large-scale release–recapture experiments using marked hatchlings to investigate the life cycle of a wild cephalopod. Based on the biological information from the recaptured cuttlefish, we validated the age

class structure inferred from the lamellae number on the cuttlebone. For each age class, growth and lifespan were estimated by examining the monthly distribution of dorsal mantle lengths reconstructed from cuttlebones collected from a fish market or coastal beaches over a period of 3 yr. Furthermore, to elucidate age-based reproductive traits of broadclub cuttlefish, gonadal maturity, presence of free ova, and mating activity of wild-caught cuttlefish including recaptured ones were also examined for each age class. Finally, we discuss the life cycle traits of this species, taking into consideration growth and reproductive characteristics of the respective age classes.

MATERIALS AND METHODS

Release–recapture experiments

In an earlier study, we evaluated the stocking effectiveness of broadclub cuttlefish hatchlings in Urasoko Bay and Kabira Bay, Ishigaki Island, Okinawa Prefecture, Japan, from 2001 to 2003 (Dan et al. 2008). In these release–recapture experiments, hatchlings were released directly into seaweed areas at 1 to 2 m depth in 2 bays (see Fig. S1 in the supplement at www.int-res.com/articles/suppl/b017p181_supp.pdf). In Urasoko Bay, 55 827 hatchlings, marked once with Alizarin red S on their cuttlebones, were released from 4 April to 13 August 2001. In Kabira Bay, to distinguish the release bay and years, 62 123 hatchlings with a double marking and 48 395 hatchlings with a single marking with Alizarin red S were released from 4 April to 2 August 2001 and from 5 April to 6 August 2002, respectively. Oka et al. (2003) released broadclub cuttlefish hatchlings with an Alizarin red S marking in Urasoko Bay and captured cuttlefish in Urasoko Bay and adjacent coastal waters, including Kabira Bay, where the hatchlings had not been released. They demonstrated that all released cuttlefish were recaptured near the Urasoko Bay, but not Kabira Bay. After the release of the hatchlings, sampling surveys using set nets were conducted in Urasoko Bay and Kabira Bay during the period from autumn 2001 to spring 2002, and in Kabira Bay from autumn 2002 to spring 2003 when cuttlefish migrate into shallow Ishigaki Island waters for breeding (Inoha 1991). Samples could not be obtained in January and February in 2003 because of the suspension of fishing operations after an accident involving a fisherman who was collecting samples. The set nets were set on nearshore reefs in the 2 bays where hatchlings were released. From 2 October 2001 to 10 June 2002, 379 wild cuttlefish and 59 marked cuttlefish released in 2001 into 2 bays were collected. In addition, from 10 September 2002 to 16 June 2003, 195 wild cuttlefish, 5 cuttlefish with a double marking released in 2001, and 32 cuttlefish with a single marking released in 2002 in Kabira Bay were collected. All marked cuttlefish were recaptured near the bay where they were released.

Collection of cuttlebones

To investigate the growth of the broadclub cuttlefish, we began to collect cuttlebones in October 2000. On Ishigaki Island, almost all broadclub cuttlefish caught by set nets or spear fishing were landed at a fish market (Fig. S1 in the supplement), and the cuttlebones were discarded there. We asked the fish market to collect and keep the cuttlebones, and 3586 were collected from 29 October 2000 to 4 April 2003. In their natural habitat, cuttlebones from dead cuttlefish drift with the current because of their buoyancy and are washed ashore. Once a month, we searched the 5 coastal beaches located on the northern part of Ishigaki Island, and collected 754 cuttlebones of this species from 30 October 2000 to 28 March 2003. This allowed collection of cuttlebones of cuttlefish too small to be landed at the fish market.

Measurement of specimens

Using the specimens collected in the earlier study (Dan et al. 2008), the dorsal mantle length (DML) and wet body weight (BW) of each cuttlefish were measured with accuracies of 1 mm and 10 g, respectively. After dissecting the animals and removing their cuttlebones, testis and spermatophoric sacs of the males, and the ovaries, nidamental glands, and accessory nidamental glands of the females were weighed to the nearest 0.01 g to determine reproductive condition. The presence of free ova in the ovary or oviduct was checked as a criterion for terminal maturation of females (Mangold 1987, Gauvrit et al. 1997). In *Sepia* species, males deposit their spermatophores onto female buccal membranes during mating activity (Hanlon & Messenger 1996, Hanlon et al. 1999, Naud et al. 2005, Wada et al. 2005). Therefore, the presence of spermatophores on female buccal membranes was checked to determine whether the female had mated or not. Alizarin markings on cuttlebones from the cuttlefish were checked macroscopically. To determine the age class of cuttlefish, the number of streaks at the

surface outcrop of lamellae along the striated zone (lamella number) (Le Goff et al. 1998, Dan et al. 2011) was counted for cuttlebones of wild cuttlefish caught from October 2001 to June 2002 (see Fig. S2 in the supplement). This could be done for 334 cuttlebones (169 males and 165 females) with good conditions for counting lamellae.

In the cuttlebones collected from the fish market or coastal beaches, we measured the striated zone length instead of the shell length, because these usually lacked the anterior thin portion of the bone and the rostrum (Fig. S2 in the supplement). The shell width and shell height were also measured to the nearest 1 mm and 0.1 mm, respectively, to reconstruct the somatic size (DML) for each sex from these measurements and the striated zone length, according to Dan et al. (2011).
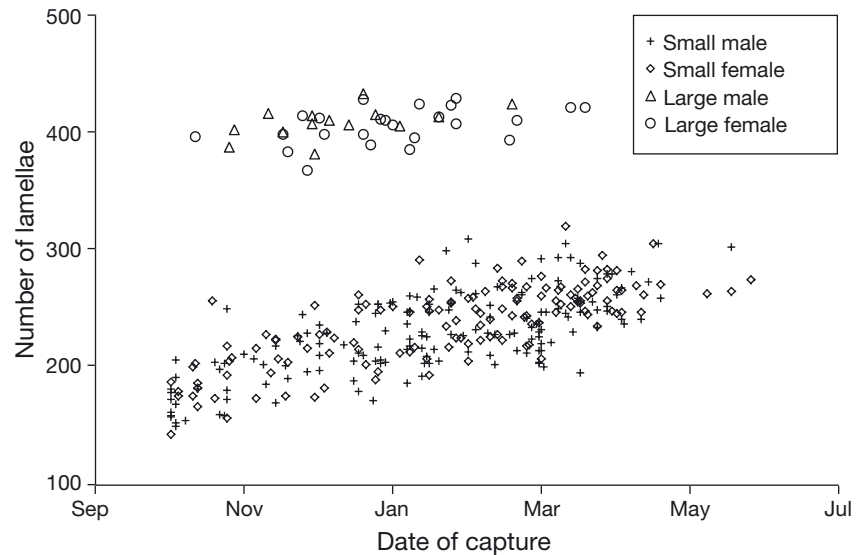

Fig. 1. *Sepia latimanus*. Seasonal distribution in the number of lamellae on cuttlebones of *S. latimanus* caught by set nets off Ishigaki Island, Japan

Age class determination and growth

In the earlier study, 2 distinct groups were isolated based on the allometric relationship between shell length and lamella number (Dan et al. 2011). We plotted the lamella number of cuttlefish against the sample collection date and detected 2 distinct groups (Fig. 1). The lamella number increased linearly throughout the fishing season in both groups (Pearson's correlation coefficient between the lamella number and the number of days from 1 August: small group, r = 0.727, p < 0.0001; large group, r = 0.420, p = 0.0094). This suggests that the increase in the lamella number may be related to age, although the rate of increase in the lamella number was different between the 2 groups (analysis of covariance, $F_{1,330}$ = 7.92, p = 0.0052). We treated these 2 groups as small and large groups, and coupled with biological information from release–recapture animals, used them to determine the age class of the specimens. First, linear discriminant analysis to distinguish the small and large groups was conducted using the day of collection (*D*, number of days from 1 August) and the DML for each sex (Fig. 1). The group discriminant index (GDI) was then applied to determine the group (age class) of other cuttlefish collected by set nets. A binomial test was used to test the null hypothesis for sex ratio for each group (H_0; sex ratio = 0.5).

The GDI was also used to separate the group of cuttlebones collected from the fish market and coastal beaches. For collected cuttlebones, sex was discriminated using the sex discriminant index (SDI) proposed by Dan et al. (2011):

$$SDI = 0.031\ SZL - 0.301\ SW + 0.499\ SH + 2.737 \quad (1)$$

where SZL is the striated zone length, SW is the shell width, and SH is the shell height. An SDI > 0 and < 0 classifies an individual as male or female, respectively. This discriminant equation was able to assign 76.3% of the animals to their sex, and the accuracy of the equation increased with increasing DML and was high (>~80%) in larger specimens (≥270 mm DML classes) (Dan et al. 2011). The DML was reconstructed for each cuttlebone using the allometric equations between SZL and DML for each sex reported by Dan et al. (2011):

$$DML = 2.617\ SZL^{0.913}\ (male)$$
$$DML = 5.989\ SZL^{0.742}\ (female) \quad (2)$$

The GDI was calculated for each sexed cuttlebone using the reconstructed DML and *D*. Although some time must pass between cuttlefish death and the body decomposition that releases the free beach-collected cuttlebones, we used the collection date without adjustment to calculate the GDI for the beach-collected cuttlebones because we had no information about the time lag between cuttlefish death and when the cuttlebones were washed ashore. We assumed this had little effect on our analysis because we collected the cuttlebones on the same beaches, predominantly during the last 10 d of each month, for 3

successive years. The number of cuttlebones (= cuttlefish) was summarized monthly according to the reconstructed DML by classified age group for each sex, and the monthly percentage abundance of each age class and the ratio of large group cuttlefish to the total number of cuttlefish was calculated during the breeding season (October to May). The monthly mean values of the reconstructed DML were calculated to infer the growth trend of each sex. Seasonal fluctuations in the monthly percentage abundance of each age class and the ratio of large group cuttlefish were evaluated with 1-way ANOVA. To investigate the effect of month, sex, and their interaction on the monthly mean DML values reconstructed from cuttlebones, 2-way ANOVA was used. The ANOVA used in this study is based on Welch's method without an assumption of the equal variances, and the different years served as replicates in the test.

Maturation and mating

As an index for reproductive condition, the gonad somatic index (GSI) was calculated as follows (Mangold 1987, Pecl 2001, McGrath & Jackson 2002):

$$\text{GSI} = (\text{GW} + \text{AGW}) / (\text{BW} - \text{GW} - \text{AGW}) \times 100 \quad (3)$$

where GW is the gonadal (testis or ovary) weight and AGW is the total of the accessory gonadal (spermatophoric sac for males and nidamental and accessory nidamental glands for females) weight. To assess changes in terminal maturation and mating associated with season and age class, the ratios of ovulated females and post-mating females to total females were calculated as ovulation rate and mating rate for monthly and age class bases, respectively. The ratio of large group females with ovulated ovaries and mating experience to the total number of females was calculated. Welch's t-test was used to compare the GW and GSI between age classes. Seasonal fluctuations in GW and GSI were evaluated with 1-way ANOVA. All statistical analyses were performed using the R language (R Development Core Team 2011), and the level of significance was assessed at $\alpha = 0.05$.

Estimation of hatchling season

Spawning of broadclub cuttlefish occurs immediately after mating (Corner & Moore 1980, Oka 1993). Therefore, mating activity represented by the mating rate can be interpreted as spawning activity, and the mating season approximately corresponds to the spawning season. However, the hatching season is not simply related to the spawning season because embryonic development depends on water temperatures, which fluctuate seasonally (Oka et al. 1989). The hatching season affects the life cycle traits of cephalopod species (Mangold 1987, Boyle & Rodhouse 2005). Therefore, we estimated the hatching season of the wild population based on the mating (spawning) season observed in the present study, and the days required from spawning to hatching (DH), which varied with temperature, were calculated using the equation reported by Oka et al. (1989) for this species:

$$\text{DH} = 1.47 \times 10^5 \text{WT}^{-2.45} \quad (4)$$

where WT is the average water temperature during the egg incubation period. We used water temperature data recorded by a data logger (Water Temp Pro, Onset Computer) every 3 h from 1 January 2002 to 31 December 2003 at a depth of 10 m in the centre of Urasoko Bay. To infer the life cycle for different age classes, the monthly abundance index of hatchlings (offspring) from each age group (AH_m) was calculated from the monthly mean values of abundance (AA_m), the monthly mean mating rate (MR_m), and the monthly mean gonad weight of ovulated females (GW_{fm}) of each age class using the following formula:

$$\text{AH}_m = \text{AA}_m \times \text{MR}_m \times \text{GW}_{fm} \quad (5)$$

RESULTS

Age class determination

The group discriminant function was estimated for broadclub cuttlefish collected from October 2001 to June 2002 as follows (Fig. 2a):

$$\text{GDI} = 0.0360D - 0.0583\text{DML} + 14.0420 \text{ (male)}$$
$$\text{GDI} = 0.0469D - 0.0830\text{DML} + 20.8420 \text{ (female)} \quad (6)$$

In this analysis, cuttlefish with a GDI >0 and < 0 were classified as small or large, respectively. The functions were able to assign 94.5 and 98.6% of the male and female cuttlefish, respectively, to the groups previously classified by lamella number (Fig. 1), showing the high accuracy of these functions. Using these discriminant functions, cuttlefish were assigned to small or large groups. The DML values of recaptured cuttlefish with Alizarin marking(s) were distributed within those of the small and large groups of wild cuttlefish (Fig. 2a,b). Considering the release season (April to August) and year
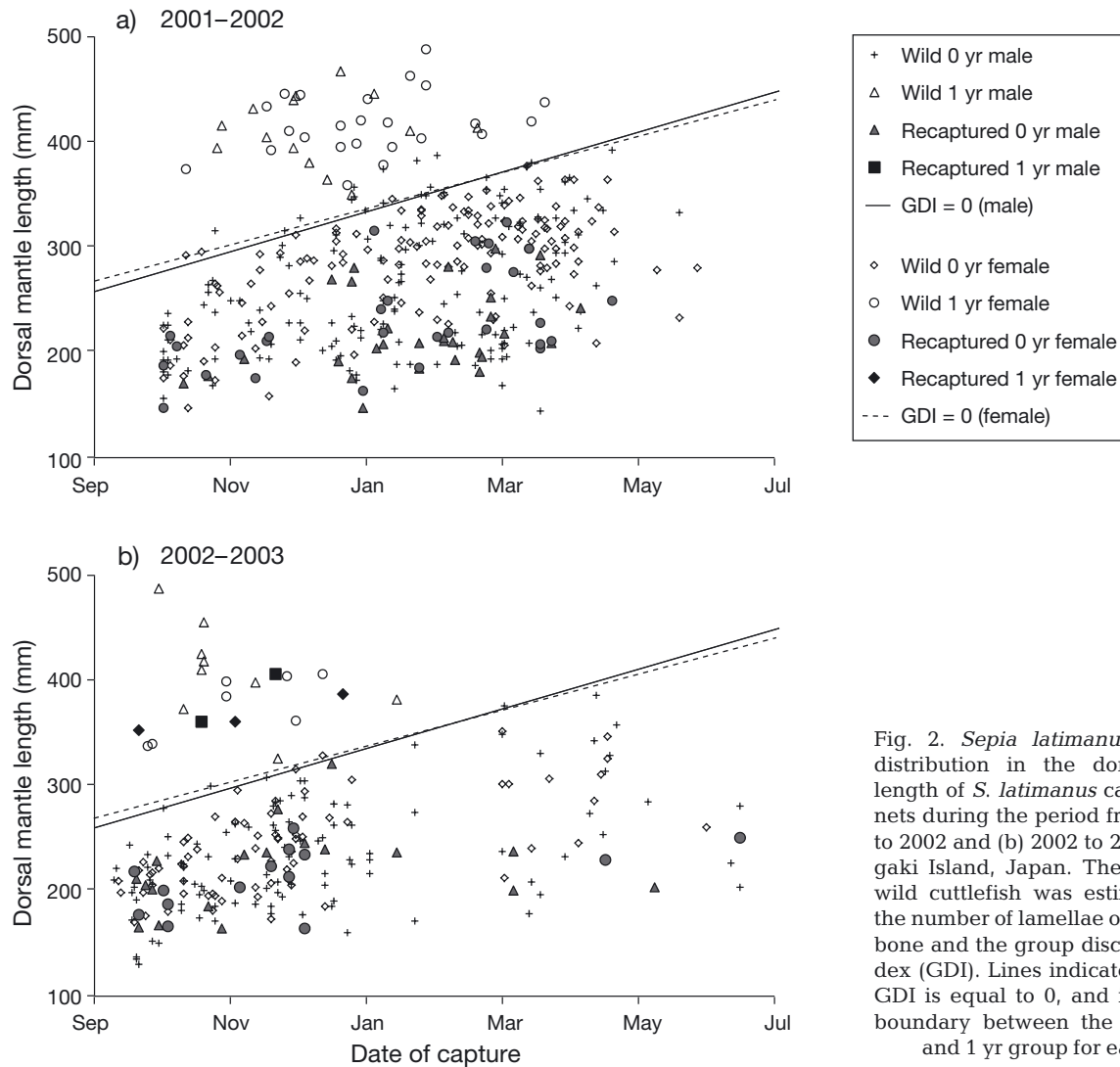
Fig. 2. *Sepia latimanus.* Seasonal distribution in the dorsal mantle length of *S. latimanus* caught by set nets during the period from (a) 2001 to 2002 and (b) 2002 to 2003 off Ishigaki Island, Japan. The age of the wild cuttlefish was estimated from the number of lamellae on the cuttlebone and the group discriminant index (GDI). Lines indicate where the GDI is equal to 0, and indicate the boundary between the 0 yr group and 1 yr group for each sex

(2001 and 2002), the ages of recaptured cuttlefish in the small and large groups were estimated to be 0+ yr and 1+ yr, respectively (Fig. 2a,b). In release–recapture experiments conducted between 1992 and 2005 (Oka et al. 2003, Dan et al. 2008), released cuttlefish were not recaptured more than 2 yr after release. Therefore, small and large groups of wild cuttlefish should be 0+ yr and 1+ yr, respectively, and these groups are defined as 0 yr and 1 yr groups in this paper.

Age class structure

In the set net surveys, 1 yr cuttlefish disappeared after March, whereas 0 yr cuttlefish were captured until June (Fig. 2). The number of 1 yr cuttlefish was smaller than that of 0 yr cuttlefish; the proportion of

1 yr cuttlefish was 8.2 and 13.8% for males and females, respectively, in the 2001 to 2002 survey, and 7.8 and 8.9% for males and females, respectively, in the 2002 to 2003 survey. No significant sex ratio bias was found in each month for each age class (binominal test, $p > 0.05$).

The approximate bimodal frequency distributions of the DML reconstructed from cuttlebones collected from the fish market or coastal beaches were assessed on a monthly basis for each sex, and they fitted well to the age groups separated by the GDI values (see Figs. S3 & S4 in the supplement at www.int-res.com/articles/suppl/b017p181_supp.pdf). Significant seasonal fluctuations were detected in the abundances of both age groups (Fig. 3) (1-way ANOVA; 0 yr, $F_{7,6.7} = 19.55$, $p = 0.0005$; 1 yr, $F_{7,6.7} = 14.93$, $p = 0.0001$). The 0 yr cuttlefish appeared in autumn (August to October), abundancy increased
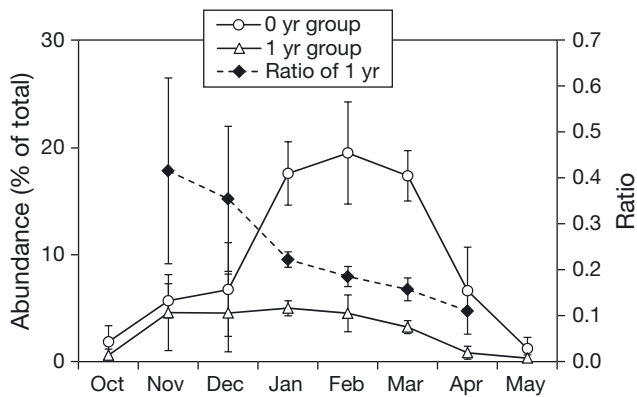
Fig. 3. *Sepia latimanus*. Monthly changes in the mean percentage abundance of 0 yr and 1 yr groups to the total number of cuttlefish during the breeding season (October to May) calculated from the length–frequency distributions of the dorsal mantle length reconstructed from the cuttlebone (see Figs. S3 & S4 in the supplement), and monthly changes in the ratio of 1 yr group cuttlefish to the total number of cuttlefish

towards winter (January to March), and thereafter decreased and they disappeared towards summer (June or July) (Fig. 3, Figs. S3 & S4 in the supplement). The 1 yr cuttlefish appeared in autumn (October), with a peak abundance from November to January, thereafter a gradual decrease towards the end of breeding season, and disappearing in May or June (Fig. 3, Figs. S3 & S4). Significant monthly changes were detected in the ratio of 1 yr cuttlefish to the total number of cuttlefish (Fig. 3); it was highest in November (0.42), and thereafter gradually decreased throughout the breeding season (1-way ANOVA; $F_{6,6.1} = 4.33$, p = 0.0048).

Growth

The mean DML value of 0 yr cuttlefish reconstructed from cuttlebones was ~180 mm in September when they initially appeared. They then grew exponentially until around December and continued to grow throughout the breeding season, reaching ~300 to 340 mm at the end of the breeding season in June (Fig. 4). No significant effects of sex and its interaction with season on the growth of 0 yr cuttlefish were detected (2-way ANOVA; sex, $F_{1,24} = 0.774$, p = 0.388; sex and month, $F_{5,24} = 0.161$, p = 0.974), whereas the effect of season was significant (2-way ANOVA; month, $F_{5,24} = 29.378$, p < 0.0001). The mean DML of 1 yr cuttlefish reached ~340 mm in August and then grew linearly, reaching ~410 to 430 mm in March to May. Significant effects of sex and season

were detected (2-way ANOVA; month, $F_{5,24} = 12.661$, p < 0.0001; sex, $F_{1,24} = 14.429$, p = 0.0009), whereas their interactive effect was not significant (2-way ANOVA; month and sex, $F_{5,24} = 0.442$, p = 0.815), indicating larger mean DML values for 1 yr males than for 1 yr females, but the same monthly growth rate.
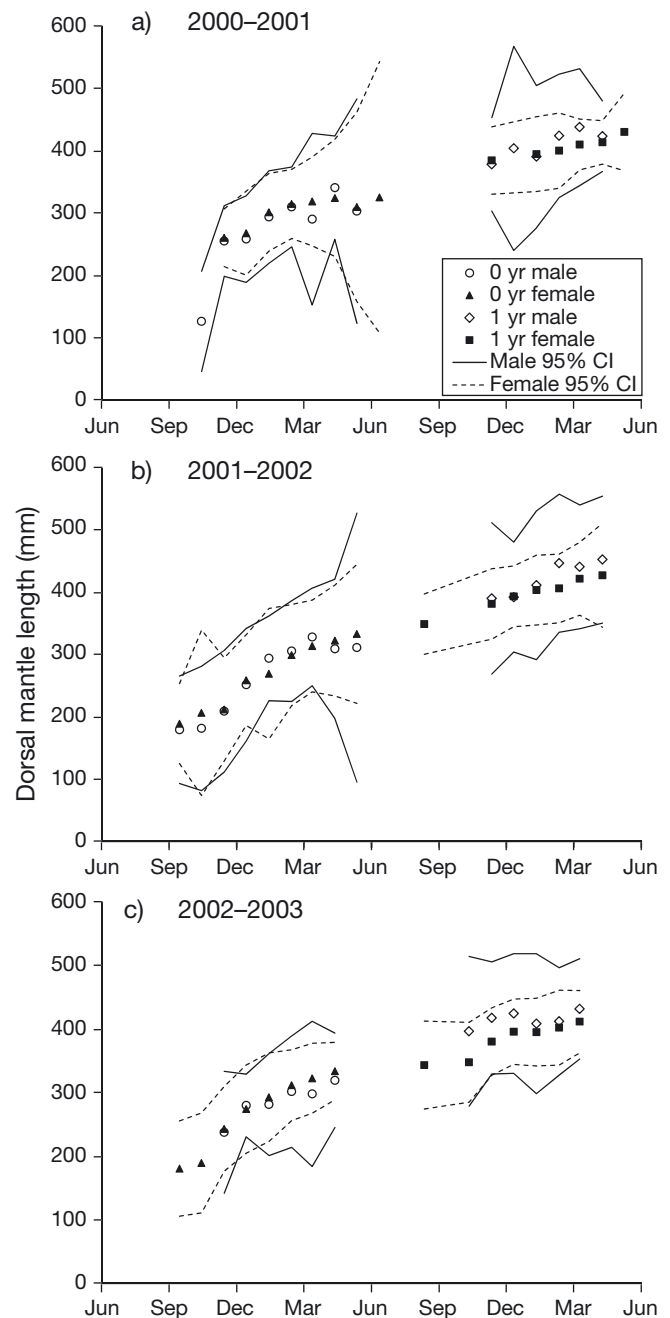

Fig. 4. *Sepia latimanus*. The monthly mean value of the dorsal mantle length reconstructed from cuttlebones collected from a fish market or coastal beaches during the period from (a) 2000 to 2001, (b) 2001 to 2002 and (c) 2002 to 2003
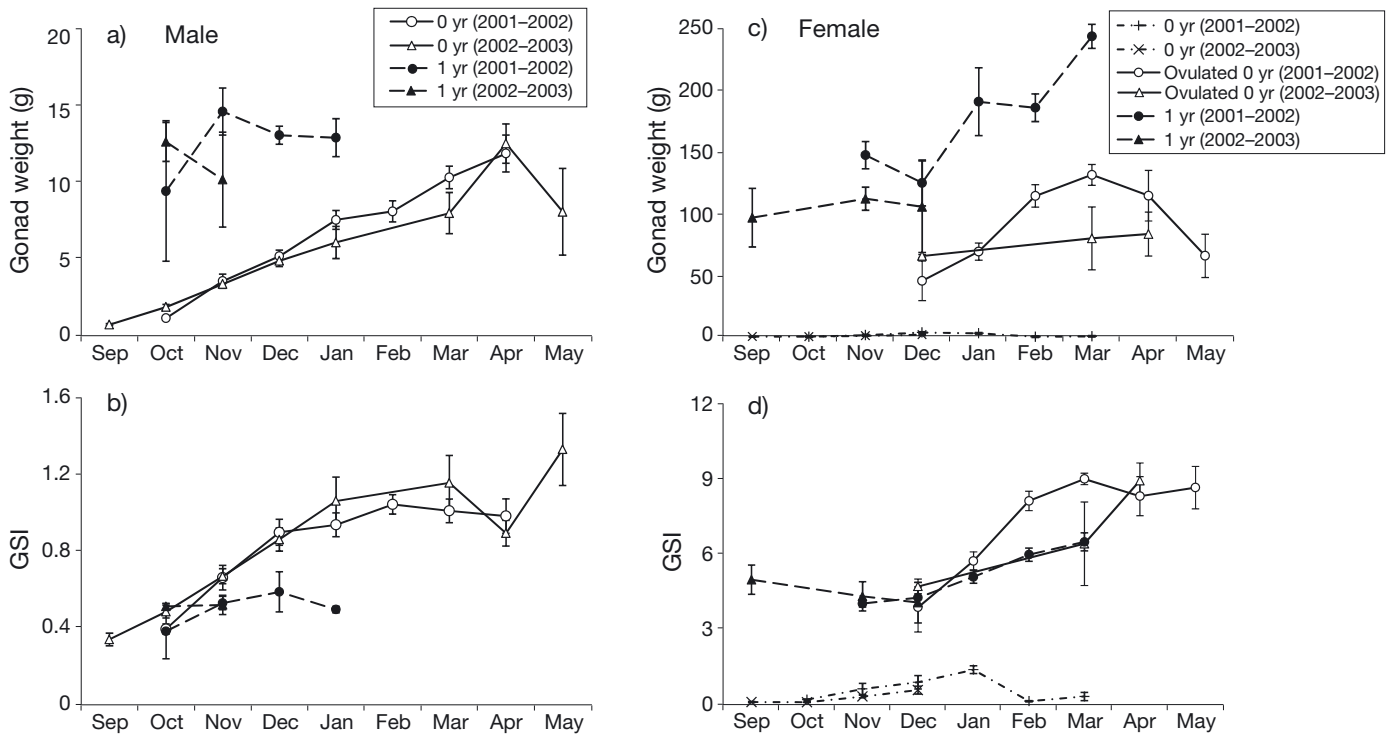
Fig. 5. *Sepia latimanus*. Monthly changes in (a) male gonadal weight (GW), (b) male gonad somatic index (GSI), (c) female GW and (d) female GSI

Maturation

The monthly changes in GW and GSI values showed that in males, 0 yr cuttlefish had lower GW but higher GSI values than 1 yr cuttlefish from October to January in both survey periods (Welch's *t*-test; p < 0.0001) (Fig. 5a,b). Significant seasonal fluctuations in GW and GSI values were not found in 1 yr males but were detected in 0 yr males (Table 1), and they tended to increase throughout the breeding season (until April or May). In females, ovulated 0 yr cuttlefish had lower GW but higher GSI values than ovulated 1 yr cuttlefish from December to March in the 2001 to 2002 survey (Welch's *t*-test; GW, p = 0.0005; GSI, p < 0.0001) (Fig. 5c,d). Significant seasonal fluctuations were detected in the GW of ovulated 0 yr and 1 yr females in the 2001 to 2002 survey, and in the GSI of all females except for ovulated 1 yr females in the 2002 to 2003 survey (Table 1). Overall trends showed that the GW and GSI increased until March for all ovulated females, but thereafter the GW decreased and the GSI was nearly constant for ovulated 0 yr females.

Table 1. *Sepia latimanus*. Results of 1-way ANOVA to compare monthly mean values of gonadal weight (GW) and gonad somatic index (GSI)

Variables	Survey period	Age class	Ovulation	df	*F*	p
Male						
GW	2001–2002	0 yr		6,55.8	79.67	<0.0001
		1 yr		3,2.6	0.36	0.7905
	2002–2003	0 yr		8,13.0	37.98	<0.0001
		1 yr		1,2.7	0.54	0.5206
GSI	2001–2002	0 yr		6,59.6	37.38	<0.0001
		1 yr		3,3.3	0.50	0.7081
	2002–2003	0 yr		8,2.3	0.03	0.8733
Female						
GW	2001–2002	0 yr	−	5,8.5	2.93	0.08124
		0 yr	+	5,14.6	8.45	<0.0001
		1 yr	+	4,5.6	10.47	<0.0001
	2002–2003	0 yr	−	4,6.3	2.93	0.1116
		0 yr	+	2,6.7	0.59	0.5784
		1 yr	+	2,1.6	0.13	0.8844
GSI	2001–2002	0 yr	−	5,7.6	11.32	0.0022
		0 yr	+	5,13.8	12.97	0.0001
		1 yr	+	4,4.3	12.89	0.0122
	2002–2003	0 yr	−	4,6.3	5.55	0.0301
		0 yr	+	2,7.6	14.14	0.0027
		1 yr	+	2,2.3	0.41	0.7053

The ovulation rate of 0 yr females showed the lowest values, around 0%, at the beginning of the breeding season (October and November); thereafter, the ovulation rate values increased linearly, reaching 100% late in the breeding season from April to May (Fig. 6a). In contrast to 0 yr females, the ovulation rate of 1 yr females showed high values of 50 to 100% from October to November, and stayed at 100% after December. In 0 yr females, the ovulation rate was lowest for females with a DML less than 200 mm; it increased with increasing DML and reached 97% for females with a DML greater than 300 mm, whereas the ovulation rate of 1 yr females showed a high value of 85%, even in the smallest size class (<400 mm) (Fig. 6b). Monthly changes in the ratio of 1 yr ovulated females to total ovulated females showed that the ratio was highest at the beginning of the breeding season (November and December) and decreased linearly to 0 in April (Fig. 6a). These results indicate that 1 yr females reached terminal maturation earlier than 0 yr females, and the num-ber of ovulated 0 yr females increased with growth throughout the breeding season, resulting in a decrease in the ratio of 1 yr females.

Mating

Post-mating females were observed at almost the same time for both age groups (18 December for the 0 yr group and 23 December for the 1 yr group in the 2001 to 2002 survey) (Fig. 7a). The mating rate values of 1 yr females increased abruptly and reached 100% in January. In contrast, the mating rate values of 0 yr females increased gradually, reaching 100% at the end of the breeding season in May. Mating was observed for females with a DML greater than 205 mm, and the mating rate tended to increase with increasing DML in both age groups (Fig. 7b). Monthly changes in the ratio of 1 yr post-mating females to total post-mating females showed that it was highest at the beginning of the breeding season (December) and decreased to 0 in April. These results indicate that almost all 1 yr females began to mate with males immediately after the onset of the mating season, whereas the number of post-mating 0 yr females increased with growth throughout the breeding season, resulting in a decrease in the ratio of 1 yr females.

Hatching season and abundance of offspring from each age class

Based on the monthly mean values of abundance, mating rate, gonad weight of ovulated females for each age class, and water temperature data for Ishigaki Island (Fig. 8), the hatching season of the wild population was estimated to extend from March to June (Fig. 9). The high hatching season appeared from April to May. The ratio of abundance of offspring from 1 yr females to the total abundance of offspring was highest at the beginning of the hatching season (maximum 0.87) and then decreased throughout the hatching season (minimum 0.22).
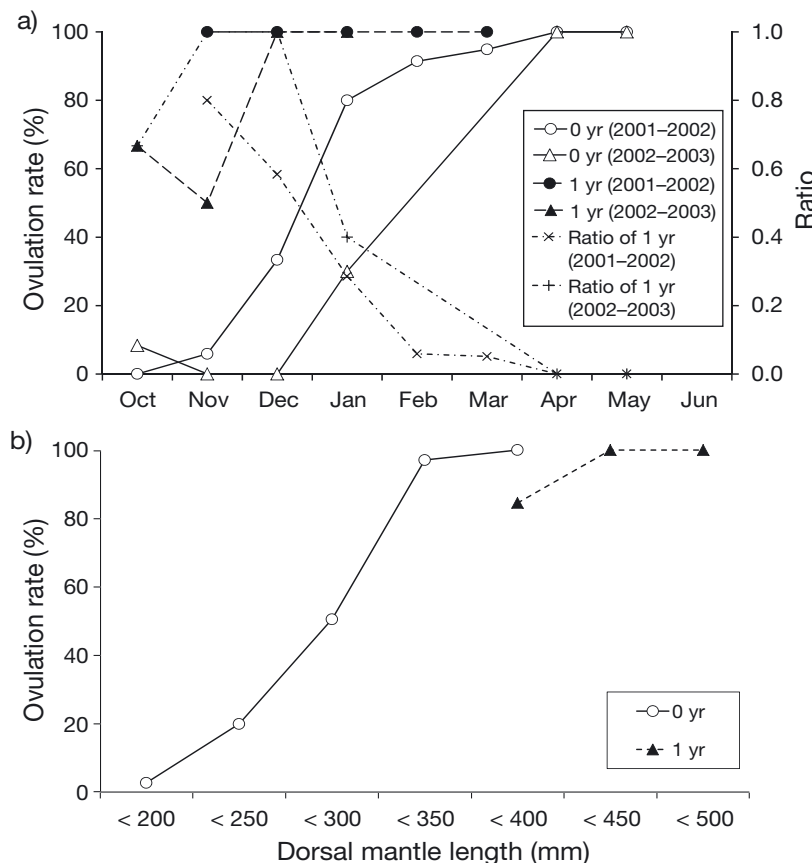

Fig. 6. *Sepia latimanus*. (a) Monthly changes in the percentage of ovulated females (ovulation rate) and the ratio of ovulated 1 yr females to the total number of ovulated females and (b) the relationship between the dorsal mantle length and ovulation rate
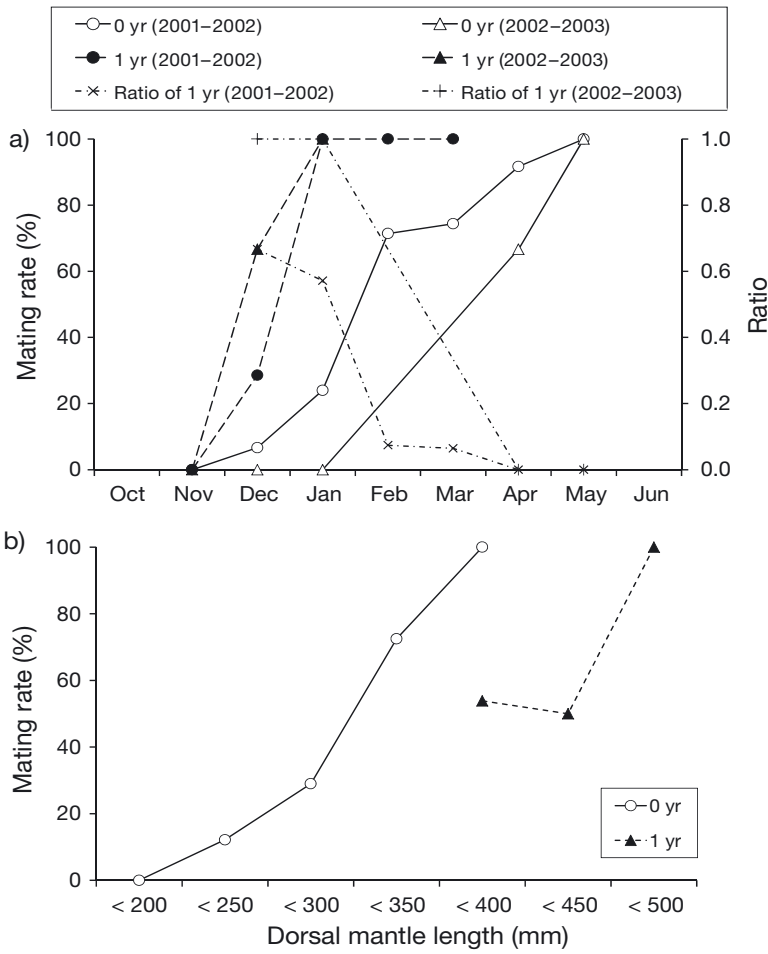
DISCUSSION

In *Sepia* species, attempts to estimate age from statoliths, which is generally used for squid, have been less successful because of poor definition of the daily increments in the statolith rings, especially for long-lived cuttlefish (Rodhouse & Hatfield 1990, Natsukari & Tashiro 1991, Bettencourt & Guerra 2000, 2001, Hall et al. 2007). There are only 2 studies that have successfully determined the age of wild-caught *Sepia officinalis* juveniles younger than 5.5 mo (Challier et al. 2002, 2005). Age group analysis based on cuttlebone morphology, such as the number of lamellae and lamellae width, has been more successful (Le Goff et al. 1998, Hall et al. 2007, Dan et al. 2011). However, the relationship between cuttlebone morphology and age has not been verified for wild cuttlefish. In the present study, broadclub cuttlefish caught by set nets were successfully separated into 2 distinct groups (small and large) by counting the number of lamellae on cuttlebones, and discriminant functions based on the DML and collection date were proposed to identify the groups. The release–recapture experiments demonstrated that, in 2 successive years, released cuttlefish were recaptured in the same bay where they were released (Oka et al. 2003, Dan et al. 2008), and confirmed that the small and large groups correspond to the 2 age groups (0 yr and 1 yr) in the present study. Furthermore, our analysis elucidated
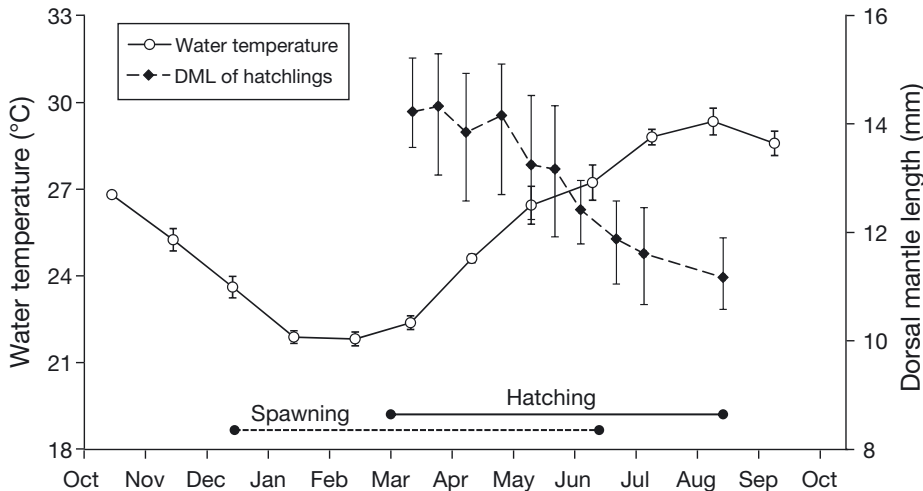

Fig. 7. *Sepia latimanus.* (a) Monthly changes in the percentage of post-mating females (mating rate) and the ratio of post-mating 1 yr females to the total number of post-mating females and (b) the relationship between the dorsal mantle length and mating rate

Fig. 8. *Sepia latimanus.* Mean (±SE) water temperature for each month from January 2002 to December 2003 at 10 m depth in Urasoko Bay, Ishigaki Island, Japan, and seasonal changes in the dorsal mantle length (DML) of hatchlings (data from Oka et al. 1989). The lower horizontal solid line and broken line show the hatching and spawning periods, respectively
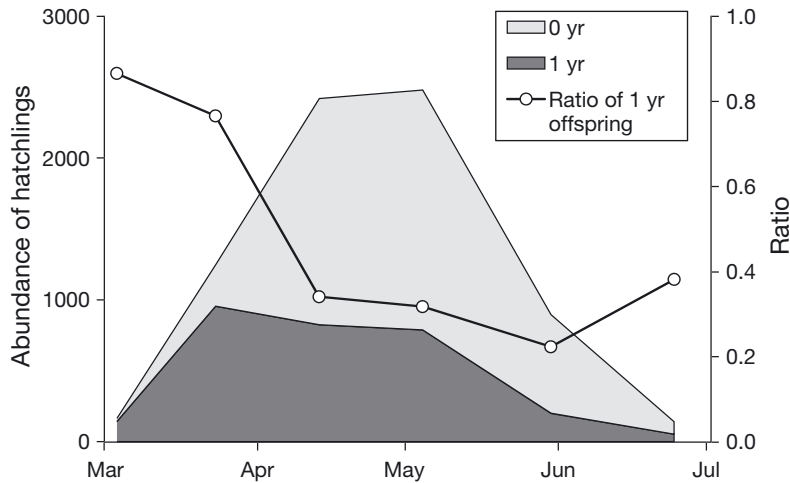
Fig. 9. *Sepia latimanus*. Seasonal changes in the abundance of hatchlings as offspring of 0 yr and 1 yr age groups, and in the ratio of 1 yr offspring to total hatchlings. Abundances were estimated by multiplying the monthly mean value of the parental abundance, mating rate, and female gonadal weight for each age group

that the length–frequency distribution of DML reconstructed from cuttlebones collected from a fish market or coastal beaches exhibited bimodal distributions corresponding to 2 age groups. These results clearly indicate that the population of broadclub cuttlefish in Ishigaki Island waters comprised 2 age groups during the same breeding season. This is the first instance for cuttlefish species in which the age class structure identified by cuttlebones and size–frequency distributions was confirmed directly by release–recapture experiments.

Coleoid cephalopods are known to have a characteristic growth pattern: they show constant growth, even in the reproductive season, over their lifespans and may all die before reaching the asymptotic size (Forsythe & Van Heukelem 1987, Rodhouse 1998, Arkhipkin & Roa-Ureta 2005). This growth pattern was observed in broadclub cuttlefish, which also showed a larger size in males than in females in the 1 yr group. This may be because of the energetic demands of reproduction (Moltschaniwskyj 2004). Forsythe & Van Heukelem (1987) suggested that cephalopod females need to divert relatively large amounts of energy to reproductive growth (such as gonadal growth) compared with males, and females generally show slower growth than males after reaching maturity.

No cases are known for coleoid cephalopods where individuals have been able to breed in several spawning seasons (Boyle & Rodhouse 2005). Yasunaga & Kajihara (1969) reported that moribund broadclub cuttlefish were frequently observed in the fishing ground late in the spawning season, suggesting post-spawning mortality. We also observed that almost all the cuttlefish broodstock used to produce the hatchlings for the release–recapture experiments, composed of 0 yr and 1 yr groups (see Table S1 in the supplement at www.int-res.com/articles/suppl/b017p181_supp.pdf), died at the end of the spawning season (54 out of 55 cuttlefish died by July in 2001 to 2002; no data for 2000 to 2001). The ovulation rate of 0 yr females reached 100% later in the spawning season, i.e. in April. This means that almost all cuttlefish examined in this study reached terminal maturation and died after spawning. Therefore, mature 0 yr and 1 yr cuttlefish are regarded as different breeding groups. Furthermore, release–recapture experiments demonstrated that these breeding (age) groups hatched in the same hatching season in the same area (bay).

Although the released hatchlings were recaptured in 2 successive years in the same bay where they were released (Oka et al. 2003, Dan et al. 2008), little is known about the habitat of 1 yr broadclub cuttlefish. Ikeda et al. (1999) measured strontium concentrations in consecutive pieces sampled from the spine to the locus of the cuttlebone of a broadclub cuttlefish (415 mm shell length in September: 1 yr group) as a proxy for ambient temperatures in the habitat, and reported fluctuations in strontium concentrations that did not agree with the seasonal fluctuation in nearshore water temperatures. They suggested that fluctuations in strontium concentrations implied migration between shallow and deep waters or between inshore and offshore benthic waters. A further report on migration was provided by Inoha (1991), who suggested that broadclub cuttlefish tend to move down to deeper areas during a high-temperature season, because they were not found in shallow waters. Accordingly, the young of the 1 yr group in the present study, which did not reach a mature size in the first spawning season, may have migrated to deeper water during higher temperatures.

Whether broadclub cuttlefish reach mature size in their first spawning season depends on the hatching time and growth rate. Temperature is known to affect the development and growth of cephalopod species (Forsythe 1993, 2004, Vidal et al. 2002). Forsythe (1993) demonstrated that small changes in temperature during the early exponential growth phase can result in marked differences in size-at-age. Around

Ishigaki Island, the water temperature rises throughout the hatching season (Fig. 8). Therefore, according to Forsythe's hypothesis, broadclub cuttlefish hatched later may take advantage of faster growth under higher temperature conditions. In contrast, Pecl et al. (2004) demonstrated that differences in hatchling size affected the size at a specific age, and hypothesized that a reduction in hatchling size caused by progressively warmer temperatures during the hatching season partially counteracts Forsythe's effect. In broadclub cuttlefish, Oka et al. (1989) reported that the mean size of hatchlings from laboratory-spawned eggs tended to decrease throughout the hatching season (14 mm in May and 11 mm in June) (Fig. 8). Therefore, the hypothesis by Pecl et al. (2004) may apply to broadclub cuttlefish; seasonal temperature fluctuations may have little effect on the growth rate of cohorts arising from any one hatching season.

The hatching time may be a more important factor affecting maturation timing of broadclub cuttlefish. Here, we infer the hatching time limit for this species to reach maturity in the first spawning season based on physiological time, i.e. degree-days from hatching to the beginning of maturation. Degree-days are calculated by multiplying the mean daily temperature above the lower developmental threshold in °C by the time in d (Begon et al. 2006), and are known to be well suited to evaluating the age at maturity in cephalopods (Wood & O'Dor 2000). The lower threshold temperature for development and growth of broadclub cuttlefish after hatching is not known. Broadclub cuttlefish spawn large-sized eggs and hatch as an adult form (Okutani 1978). Therefore, we considered that the threshold temperature of development was similar between egg (embryo) and young after hatching, and we used the value of 14.9°C estimated from a function for the relationship between the number of days required from spawning to hatching and water temperature reported by Oka et al. (1989) (see Fig. S5 in the supplement at www.int-res.com/articles/suppl/b017p181_supp.pdf for an estimation of the threshold temperature for egg development). We calculated the degree-days required from hatching to 18 December when the earliest ovulated 0 yr female was observed, to 24 March when the last non-ovulated 0 yr female was observed, and to 23 December in the following year when the earliest ovulated 1 yr female was observed. The hatching season of the wild broadclub cuttlefish was estimated to extend from March to June (Fig. 9). In captive cuttlefish, the beginning of the hatching season was delayed until April (see Fig. S6 in the

supplement) because of delayed spawning (egg collection), which started in January (Dan et al. 2008), compared with that of the wild counterparts, which began in December. In the wild cuttlefish, the hatching season was estimated to end in late June, whereas the hatching season was prolonged to July/early August in captive cuttlefish (Fig. S6 in the supplement). Fishing activity for wild cuttlefish terminated in May (Fig. 2) because of low catches. Therefore, wild cuttlefish may spawn after May and their hatching period may extend to July/early August. Our analysis estimated that broadclub cuttlefish, which hatched at the beginning of the hatching season (3 March), require 3362 degree-days to reach the earliest terminal maturation on 18 December, and cuttlefish hatched prior to 18 May can satisfy this threshold value by the latest terminal maturation on 24 March (Fig. 10). This implies that cuttlefish hatched before mid-May may reach breeding condition in the hatching year as the 0 yr age group, while those hatched after mid-May may not reach breeding condition in the hatching year, but return as recruits the following breeding season as the 1 yr age group. Degree-day values until maturation of 1 yr females were large compared with those of 0 yr females. This is probably because of the effect of photoperiod on gonadal maturation (O'Dor et al. 1977, Mangold 1987, Le Goff & Daguzan 1991). Furthermore, it should be noted that the degree-day values for 1 yr females might have been overestimated because the young of the 1 yr
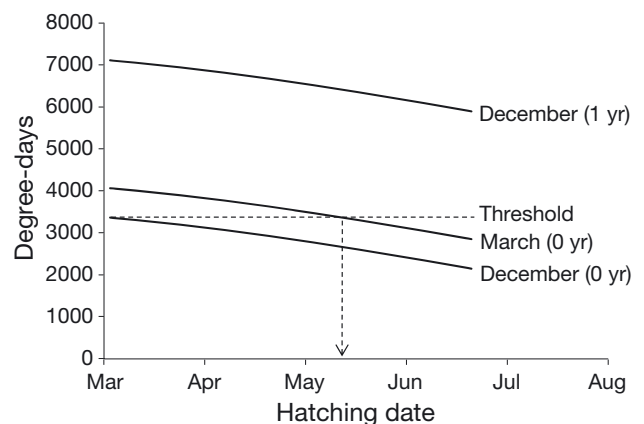

Fig. 10. *Sepia latimanus*. Relationships between the hatching date and totalized degree-days from hatching to the earliest terminal maturation of 0 yr cuttlefish (18 December), to the latest terminal maturation of 0 yr cuttlefish (24 March), and to the earliest terminal maturation of 1 yr cuttlefish (23 December). The horizontal broken line shows the lowest threshold of degree-days required from hatching to terminal maturation of 0 yr cuttlefish. The arrow with a broken line indicates the theoretical latest hatching date permitting terminal maturation of 0 yr cuttlefish

group may have migrated to deeper water with lower temperatures, as discussed above. Taking the hatching season and capture date of both age groups into consideration, the maximum lifespan of broadclub cuttlefish is estimated at 16 and 25 mo for 0 yr and 1 yr groups, respectively.

The analysis of ovulation rate and mating rate indicated that 1 yr and 0 yr females were major spawners in the early and late breeding seasons, respectively. Reproductive traits indicating that 1 yr cuttlefish are early breeders may be inferred for males, because larger males are known to be superior to smaller ones in acquiring females for mating (Corner & Moore 1980). Based on the abundance of offspring from each age group estimated by reproductive traits of both breeding groups (Fig. 9), 0 yr cuttlefish offspring may have originated equally from 0 yr and 1 yr females, while the ratio of offspring origin was biased towards 1 yr females in the early hatching season. Furthermore, 1 yr cuttlefish mainly originated from offspring hatched from 0 yr females. Therefore, broadclub cuttlefish appear to alternate partially between short and long life cycles.

A life cycle model that has alternating short and long life cycles attributed to different hatching seasons and reproductive traits of large and small females has previously been proposed for *Sepia* species (e.g. *Sepia officinalis*, Mangold 1966, von Boletzky 1983, Le Goff & Daguzan 1991, Gauvrit et al. 1998; *Sepia apama*, Hall et al. 2007). However, the age and hatching place of the 2 breeding groups have not been clarified in any studies. In this study, we highlighted the alternating life cycle traits more quantitatively based on age determination techniques and release–recapture experiments. *Sepia officinalis* is thought to exhibit variation in its life cycle across its geographic range (Mangold 1966, von Boletzky 1983, Gauvrit et al. 1997, 1998, Dunn 1999, Guerra 2006). Broadclub cuttlefish are distributed throughout the tropical and subtropical waters of the Indian and western Pacific Oceans (Reid et al. 2005). Therefore, life cycles of this species may vary with geographic distributions. To elucidate the development of such a flexible life cycle strategy in cuttlefish species, it is important to clarify the age and hatching area of each breeding group for different life cycle traits. Release–recapture experiments using hatchlings with marked cuttlebones can provide reliable information to improve knowledge regarding age and distribution of cuttlefish. Moreover, further studies to determine the hatching season (months) of cuttlefish using marked hatchlings will further confirm the alternating life cycle for cuttlefish species.

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