Spawning pattern of the neon flying squid
Ommastrephes bartramii (Cephalopoda: Oegopsida)
around the Hawaiian Islands

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Summary: The neon flying squid, Ommastrephes bartramii, is an oceanic squid species that is widely distributed in the North Pacific, with the winter-spring cohort spawning around the Hawaiian Islands. Here, we investigated the spawning characteristics of O. bartramii by analyzing various reproductive parameters of individuals (622 males, 108 females) collected in this region. Female spawning status was determined from the somatic indices and histological characteristics of the ovaries. At all developmental stages, the ovaries of spawned females contained oocytes, and oviduct fullness was not correlated with body size. Thus, because the eggs mature asynchronously, with multiple filling and evacuation events, this species is considered an intermittent spawner. Mature males with developed accessory glands were also present within the distribution range of healthy spawned females, indicating that mating occurs between spawning events. Our data indicate that the first spawning event occurs at a mantle length of ~520-540 mm for Hawaiian O. bartramii. Subsequently, the squid forage and grow, and refill the oviducts, before the second spawning event occurs.

Keywords: North Pacific; squid; Ommastrephes bartramii; reproductive strategy; spawning pattern.

INTRODUCTION

All living cephalopods are considered semelparous (i.e. characterized by a single reproductive event; Boyle 1983, Calow 1987, Rodhouse 1998) with the only exception being Nautilus, which has a life span of more than 20 years (Rocha et al. 2001). However, recently, several authors (Nigmatullin et al. 1996, Nig-
matullin 2002, Nigmatullin 2011) have reported complex reproductive patterns that cannot be categorized as semelparous, particularly in tropical and subtropical oegopsid squids belonging to the family Ommastrephidae. For example, intermittent spawning without somatic growth between spawning events has been reported for Illex coindetii (González and Guerra 1996), Todaropsis eblanae (Rasero et al. 1995) and Illex illecebrosus (O’Dor and Dawe 1998). Furthermore, intermittent spawning with somatic growth between spawning events has been reported for Ommastrephidae (Reznik and Bessmertnaya 1993, Nigmatullin and Laptikhovsky 1994, Nesis 1996, Laptikhovsky and Nigmatullin 2005, Nigmatullin and Markaida 2009). Recently, iteroparity has also been reported in the squid Kondakovia longimana (Laptikhovsky et al. 2013).

The neon flying squid, Ommastrephes bartramii, is an oceanic squid that occurs in subtropical and temperate waters worldwide (Roper et al. 2010). The population in the North Pacific includes an autumn spawning cohort and a winter-spring spawning cohort (Yatsu et al. 1998). Individuals live for one year, migrating between the spawning grounds in tropical waters and foraging grounds in sub-Arctic waters during this period (Bower and Ichii 2005, Chen and Chiu 2003, Murata and Nakamura 1998, Yatsu et al. 1998). The main spawning and nursery ground of the autumn cohort occurs in the subtropical frontal zone (STFZ) of the north Pacific Ocean. This location is characterized by enhanced productivity in winter because of its proximity to the transition zone chlorophyll front (TZCF). In comparison, the spawning and nursery ground of the winter-spring cohort occurs within the subtropical domain (STD), which is less productive (Ichii et al. 2009). The STFZ is defined by a salinity range of 34.6 to 35.2 at the sea surface, generally occurring at ~29 to 34°N latitude, with the STD occurring to the south of the STFZ (Ichii et al. 2009, Roden 1991). O. bartramii are considered to be paralarvae (i.e. young cephalopods in the planktonic stages) from hatching to one month of age (Bigelow and Landgraph 1993), juveniles and subadults from one month of age until they reach a mantle length (ML) of ~25 cm (Ichii et al. 2009), and adults above 25 cm ML.

The oocytes develop in the ovary, which extends from the posterior end of the digestive gland to approximately the posterior end of the mantle. Ova are released from the ovary and accumulate in the oviducts. The nidamental glands produce the outer layer of the egg mass (Bower and Sakurai 1996). Females store viable sperm in the buccal area, so mating and spawning do not need to coincide (Harman et al. 1989). Closely related Dosidicus gigas and Sthenoteuthis pteropus ommastrephid females spawn in pelagic waters, producing spherical egg masses (Laptikhovsky and Murzov 1990, Staaf et al. 2008). For two O. bartramii collected from the Atlantic ocean Laptikhovsky (2011) calculated a potential fecundity of 4.9 and 3.7 million eggs. The sperm duct of males transfers the sperm from a testis that is located to the anterior of the spermatophoric organ, where spermatophores are formed. Spermatophores are tubular structures stored in the spermatophoric sac (Needham’s sac), which opens into the mantle cavity through the penis (Jereb and Roper 2010). In cephalopods, allometric growth relationships between somatic, reproductive, and digestive components change during ontogeny, depending on the state of maturity and nutritional status (Boyle and Rodhouse 2005). These allometric growth relationships may provide a way of directly determining the spawning status of neon flying squid individuals that are collected from the sea.

In this study, we aimed to examine the reproductive characteristics of the winter-spring cohort of O. bartramii near the Hawaiian Islands, using individuals collected from 1990 to 2000 and 2013. The findings of this study, combined with the results of previous studies, are expected to clarify whether this species invests in single or multiple spawning.

MATERIALS AND METHODS

Sampling

Squid were collected near Hawaii aboard R/V Hokusei Maru (Hokkaido University, Japan) and R/V Kaiyo Maru (Fisheries Research Agency, Japan). Sampling aboard R/V Hokusei Maru was performed by manual jigging, gillnetting, and trawling on 11 cruises from February to March every year from 1990 to 2000, between the latitudes of 20 and 35°N and the longitudes of 150 and 180°W. Collection aboard R/V Kaiyo Maru was conducted in December 2013 in the sea to the north of Hawaii, between the latitudes of 33 and 24°N and the longitudes of 173 and 155°W. A total of 719 (622 males, 97 females) individuals were collected by R/V Hokusei Maru (from 78 stations), in addition to 11 individuals (all females) by R/V Kaiyo Maru (from 8 stations). All squids were used in the analysis. Monthly data from the 1990-2000 survey years (excluding 2013) were combined because interannual variation in ML is thought to be minimal (Murata and Hayase 1993, Yatsu et al. 1998).

Analysis of the reproductive system

For the analysis, we selected female squid that had an ML >400 mm and male squid that had an ML >300 mm, to ensure that only mature squid were included in the analysis. Dorsal ML (to the nearest 1 mm) was measured, and the following parameters were estimated: body weight (to the nearest 1 g), stomach weight, and digestive gland weight (Laptikhovsky and Nigmatullin 1992, 1993). We obtained the testis weight and accessory gland weight of males (Nigmatullin et al. 2003). We also obtained the ovary weight, oviduct weight, nidamental gland length, nidamental gland weight, and mating status (copulated or not) of females. Just 11 of the female squid collected by R/V Kaiyo Maru were used for the analysis of oocyte size and potential fecundity (PF). To determine the number of oocytes in each ovary, we collected three subsamples (100 mg) from the ovary surface, ovary core and intermediate layer. Each subsample was weighed and
then observed in a Bogorov chamber under a binocular microscope (Nigmatullin and Markaida 2009). PF was calculated as the sum of total oocyte (>0.05 mm in diameter) number in the ovary and total egg number in the oviducts (Nigmatullin et al. 1995, Nigmatullin 1997, Laptikhovsky and Nigmatullin 1999, Nigmatullin and Laptikhovsky 1999). Oviducal load (OL) is the number of eggs in the oviducts (Nigmatullin and Markaida 2009).

We used the allometric growth relationships of somatic and reproductive components to determine the spawning status of *O. bartramii* directly. Specifically, we calculated and analyzed the somatic indices of female squid and the volume of oocytes in the ovaries to determine spawning status. The somatic indices used in this study were calculated using the definitions shown in Table 1. Reproductive maturity was assessed on the basis of the maturity stages I to VII proposed by Nigmatullin (1989). These stages were adjusted for the neon flying squid, whereby stages I and II were defined as immature, stage III as early maturing, stage IV as late-maturing, stage V as mature, stage VI as spawning state (after the first spawn), and stage VII as true spent. Stage VII squid were not collected during any of the cruises. Statistical analyses were performed using R software (R Core Development Team 2013).

**RESULTS**

**Somatic indices**

The somatic indices of 622 males and 97 females (1990-2000 data) were analyzed. Females (ML: 526±4.5 mm, mean±SE) were larger than males (ML: 333±0.7 mm). All females with ML >400 mm had eggs in the oviducts, indicating that all individuals had attained stage IV sexual maturity or above. Another indicator of sexual maturity in females is the relative size of the nidamental glands (Durward et al. 1979, Okutani and Tung 1978). The nidamental glands of mature females were relatively large, opaque, and white. The glands ranged in size from 68 to 190 mm, and represented 0.9% to 4% of the total body weight in individuals >400 mm ML. The nidamental gland index differed significantly among maturity stages IV, V, and VI (P<0.01, analysis of variance [ANOVA]; Fig. 1). Nidamental gland weight increased from the late-maturing stage to the mature stage and decreased after spawning (spawning state). Nidamental gland weight was positively correlated (r=0.56, P<0.01) with ML. Within each of the three stages (IV, V, and VI), neither ML (F_{2,83}=1.8, P>0.05, ANOVA) nor body weight (F_{2,83}=2.9, P>0.05, ANOVA) differed significantly. Stage VI females ranged in size from 516 to 572 mm ML.

The oviduct index (ODI) distribution of females was scattered (Fig. 2). This index showed no correlation with ML (Spearman rank coefficient r=0.148, P>0.05) in females with ML >400 mm. Although there was considerable scatter in ODI among mature individuals, there was no evidence that any female was dying. All mature females had food in the stomach. In addition, the weight of the stomach content in stage VI
females (spawning state) was similar to that of stage IV and V females ($F_{2,74}=1.85$, $P>0.05$, ANOVA; Figs 3 and 4). The ovary index was also not correlated with ML (Spearman rank coefficient $r=0.031$, $P>0.05$). Female squid of advanced maturity stages had large ovaries (ovary somatic index [OSI]: 4.34±0.83%, Figs 4 and 5, Table 2).

There was a negative correlation between the testis index and ML (Spearman rank coefficient $r=-0.48$, $P<0.001$) of males. However, there was no significant correlation between the accessory gland index and ML (Spearman rank coefficient $r=-0.04$, $P>0.05$) of males.

Oocyte size and fecundity

The oocytes in the ovaries of mature females were at various stages of development, indicating asynchronous development. It was possible to distinguish six size groups of oocytes that corresponded to the stages of oocyte development (after Nigmatullin et al. 1995). Size group I corresponded to the second phase of provitellogenesis, in which oocytes were polygonal and the centrally situated nucleus was oval and large. Group II corresponded to the third phase of provitellogenesis. Oocytes were either oval or goblet-shaped and the cytoplasm volume was greater than that of the

Table 2. – Mean±SD and range values for the size (mantle length, ML) and weight (body weight, BW) of female *Ommastrephes bartramii*. Mean values are shown for the stomach content index (SCI), ovary somatic index (OSI), nidamental gland somatic index (NSI), and Hepato somatic index (HSI).

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>ML (mm)</th>
<th>BW (g)</th>
<th>SCI</th>
<th>Mean indices (%)</th>
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<tr>
<td></td>
<td>Min.</td>
<td>Mean±SD</td>
<td>Max.</td>
<td>SCI</td>
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<tr>
<td>VI</td>
<td>416</td>
<td>493.6±53.9</td>
<td>572</td>
<td>2165</td>
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<tr>
<td>V</td>
<td>415</td>
<td>507±61.2</td>
<td>595</td>
<td>2260</td>
</tr>
<tr>
<td>IV</td>
<td>420</td>
<td>522.1±54.25</td>
<td>600</td>
<td>2100</td>
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</table>
preceding size group. Group III corresponded to the intercalary period of oocyte development. Oocytes were leaf-like, of dark colour, with numerous shallow, longitudinal grooves together with follicle cells that protruded into the grooves. The nucleus was not visible in this group. Group IV corresponded to the first and second phases of trophoplasmatic growth. Oocytes were covered with reticulate grooves, and were dark in colour. Group V corresponded to the third phase of trophoplasmatic growth. Oocytes were rounded and yellow and the reticulate grooves had almost disappeared. Group VI corresponded to the fourth phase of the trophoplasmatic growth. Oocytes had a smooth surface and were oval. Data on minimum and maximum size of oocytes are given in Table 3. The percentage of oocytes belonging to different size groups changed as the reproductive system developed (Fig. 6). In immature females, oocytes of size group I were prevalent (76%), with the remainder belonging to size groups II and III. Ripe oocytes were absent in maturing females. Mature and spawning state females always had group I oocytes, but with different percentages (Fig. 6). The relative number of ripe oocytes (group VI) was always low (<1%) in mature and spawning state females, because they are immediately transferred into the oviducts after extrusion into the coelom. Individual PF varied considerably, ranging from 7 million oocytes in immature females to 2.4-7.0 million oocytes in maturing and mature females (Table 4).

**DISCUSSION**

**Structure of the reproductive organs**

The results of this study indicate that *O. bartramii* is an intermittent spawner that exhibits somatic growth between spawning events, as previously reported for *S. oualaniensis* (Harman et al. 1989), *T. rhombus* (Nigmatullin et al. 1995), and other higher ommastrephids (Reznik and Bessmertnya 1993, Nigmatullin and Laptikhovsky 1994, Zuyev et al. 2002, Laptikhovsky and Nigmatullin 2005, Nigmatullin 2011). The primary evidence supporting this suggestion is the lack of a strong correlation between body size and the number of mature oocytes in mature females, coupled with proof of the
occurrence of multiple filling and evacuation events in the oviduct. Ova are stored in the oviducts; hence, the fullness of the oviducts may be used as an indicator of repeated spawning. However, the fullness of the oviducts was not statistically correlated with body size in our study. Single spawners should show a gradual increase in oviduct fullness as size increases (Young et al. 1997), but this trend was not detected in the present data. Variability in oviduct fullness presents convincing evidence of batch spawning in females that grow substantially after reaching sexual maturity and continue to produce mature oocytes throughout this growth period.

*O. bartramii* females reach sexual maturity at ~300 mm ML (Yatsu et al. 1998, Brunetti et al. 2006, Li et al. 2011), after which spawning occurs under favourable conditions. Because we did not find any sexually immature females with ML >400 mm, substantial growth must occur in females after attaining maturity (Yatsu et al. 1998, Li et al. 2011). A positive correlation is expected between ML and oviduct fullness (ODSI) for single spawning squids (e.g., *T. pacificus*, Ikeda et al. 1993), because the oocytes are produced and then develop in the ovaries before being transferred to the oviducts at stage VI for storage until spawning (Moltchanowski 1995). However, the lack of correlation between ML and ODSI obtained in this study indicates that oocytes are present in the oviduct throughout stages IV, V, and VI. The OSI value showed the presence of oocytes in the ovaries of females with mature ML ranges (400-600 mm). This finding indicates that all stages of oocytes may be simultaneously present in the oviduct. The somatic indices of 11 females at three maturity stages (late-maturing, mature, and spawning state) collected from a single station (year 1992) are presented in Figure 4. For these females, the OSI value remained within a narrow range (2.5-4.0). Mature squid had high oviduct content but minimal stomach content (stomach content index [SCI], 0 to 0.2). In contrast, spawning state squid had smaller oviduct content but substantial stomach content (SCI, 1 to 4). Spawning state squid also had high (6.58±2.05) HSI values showing the active digestive system. Thus, female *O. bartramii* seem to grow after reaching maturity, and continue to produce eggs throughout this period. Therefore, oocytes are expected to accumulate in the oviducts, unless spawning occurs (Moltchanowski 1995). However, in the present study, oviduct fullness and ovary fullness (OSI) were generally unrelated to body size. This result is indicative of batch spawning. The presence of oocytes of all maturity stages indicates that oocytes are produced continuously and not in a single batch. After stomach content analysis, females appeared to forage irrespective of ML and maturity stage (Figs 3 and 4). These results indicate that the neon flying squid develops ova continuously, and not in a single batch. After stomach content analysis, females appeared to forage irrespective of ML and maturity stage (Figs 3 and 4). These results indicate that the neon flying squid develops ova continuously, and not in a single batch. After stomach content analysis, females appeared to forage irrespective of ML and maturity stage (Figs 3 and 4). These results indicate that the neon flying squid develops ova continuously, and not in a single batch. After stomach content analysis, females appeared to forage irrespective of ML and maturity stage (Figs 3 and 4). 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Spawning pattern

The mean size of a neon flying squid at maturity is ~300-330 mm ML for males and 370-570 mm for females (Yatsu et al. 1998). Furthermore, females tend to be larger than males of the same age (Ichii et al. 2009). Yatsu et al. (1998) reported a peak in oviduct fullness for O. bartramii: ~540 mm ML. This finding may indicate the size at which neon flying squid first spawn. Young et al (1997) also reported asynchronous ovulation in O. bartramii based on the size-frequency distribution of oocytes in the ovaries of 12 squids. From the results of these previous studies and our own findings, we hypothesize that the first spawning event in the neon flying squid occurs at ~520-540 mm ML (Fig. 7). Subsequently, the female feeds, undergoing somatic growth, and refills the oviduct. The second spawning event commences after the female has again passed through maturity stages IV and V (Fig. 7). Since our sample size was small, for calculating exact somatic growth between spawning events future studies are required. Multiple spawning has also been reported for related ommastrephids, including S. oualaniensis (Harman et al. 1989), S. pteropus (Laptikhovsky and Nigmatullin 2005), and Ornithoteuthis antillarum (Arkhipkin et al. 1998). Ichii et al. (2009) suggested that the extended spawning season of the neon flying squid might represent a risk-spreading strategy to reduce the chance of population collapse. The extended spawning season is achieved by dispersing the population through numerous seasonal cohorts and, possibly, through the occurrence of multiple spawning events. The lack of correlation between the body size and the accessory gland somatic index in males indicates that males continuously produce spermatozoa. In the current study, these mature males were scattered throughout the distribution range of healthy spawning state females. Thus, it is possible that mating occurs between spawning events.

Based on our findings, we suggest that the spawning pattern of O. bartramii is similar to the pattern proposed for T. rhombus (Nigmatullin et al. 1995), D. gigas (Nigmatullin and Markaida, 2009), and both Sthenoteuthis species (Nigmatullin and Laptikhovsky 1994, Zuyev et al. 2002, Laptikhovsky and Nigmatullin 2005). We suggest the following life-history model for female O. bartramii. Specifically, the maximum number of oocytes forms by the end of stage II of maturity in immature individuals. At subsequent developmental stages, the total number of oocytes remains constant, but they start to develop asynchronously. As soon as the oocytes ripen, they are released from the follicles into the sexual coelom and are transferred to the oviducts, where they accumulate. At the same time, the nidamental and oviduct glands develop. When the oviducts are full of ripe eggs, a spawning event takes place, as long as the SST range is favorable (21 to 25°C).

Spawning strategies

Because of the high levels of PF, the presence of primary and secondary oocytes in the ovary of mature females indicates that O. bartramii invests in intermittent spawning, as described for T. rhombus (Nigmatullin et al. 1995), D. gigas (Nigmatullin and Markaida, 2009) and both Sthenoteuthis (Nigmatullin and Laptikhovsky, 1994, Zuyev et al. 2002, Laptikhovsky and Nigmatullin 2005). In other words, feeding occurs between spawning events (i.e. during the interval between each successive filling and evacuation event in the oviducts) at the spawning ground, fueling both somatic growth and new oocyte development. In a lifetime they might release just two or three large egg masses, and possibly spawning rates vary individually. Future studies should focus on determining the duration of maturity, the rate at which the oviducts fill between spawning events, and the length of intervals between spawning events. The spawning/nursery ground for the winter-spring cohort (subtropical domain) is an oligotrophic region where the flux of nutrients into the euphotic zone is probably the lowest of any oceanic environment (Cullen 1982, Eppley and Peterson 1979). Batch spawning may represent an adaptation to highly unstable environments, where a high rate of paralarval survival depends on favorable, but fortuitous and temporary, oceanographic conditions (Rocha et al. 2001). We subscribe to the concept proposed by Harman et al. (1989), whereby batch spawning might be a common strategy among tropical and subtropical squids.

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REFERENCES


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