

# Hyposalinity and incremental micro-zooplankton supply in early-developed *Nemopilema nomurai* polyp survival, growth, and podocyst reproduction

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**ABSTRACT:** Early-developed polyps of the giant jellyfish *Nemopilema nomurai* that are sexually produced during the rainy season form the principal recruitment for benthic population stages, regulating the mass outbreaks of medusae that occur in east Asian marginal seas. Their survival, development and subsequent asexual reproduction are likely facilitated by hyposaline seawater and potentially enhanced micro-zooplankton around the estuaries that comprise the major nurseries of medusae. In this study, 8-tentacled polyps that developed from planulae were incubated at 11 salinities from low to high (8, 11, 13, 15, 18, 20, 23, 25, 28, 31, 33) with 3 feeding frequencies (once every 3 d, once every 9 d, unfed; *Brachionus plicatilis* concentration: 0.16 mg C l<sup>-1</sup>) at a constant temperature (19°C). Survival rate of early-developed polyps increased 3-fold at salinities 11 to 20, >80% greater than at salinities 25 to 33. The composition of fully developed polyps, somatic growth, and podocyst diameter positively depended on food supply, except at salinity 8. The potential podocyst production of polyp colonization reached normal peak at salinity 20 in the group that was fed once every 3 d. Excystments were significantly restricted at salinity <20. These findings confirm that estuarine areas with salinities from 11 to 20 are appropriate for *N. nomurai* polyp colonization, where prospectively increasing micro-zooplankton supply rooted in frequent eutrophication may benefit polyps. An asexual reproduction strategy also corresponded with autumn salinity fluctuations. The intensity of diluted water and monsoonal rainfall, as well as plankton supply around the estuaries in autumn may fundamentally affect polyp abundance and size, determining the population size of medusae in the following spring.

**KEY WORDS:** Polyps · Low salinity · Survival · Growth · Podocyst reproduction

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## INTRODUCTION

*Nemopilema nomurai*, one of the largest jellyfish in the world with an observed maximum umbrella diameter of 2 m and wet weight of 200 kg (Omori &

Kitamura 2004), has been attracting the concern of scientists recently due to the potentially deleterious impacts of massive blooms on human social stability, economic development, and marine ecosystem balance in east Asian marginal seas (Uye 2014). Previ-

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ous outbreaks of *N. nomurai* medusae occurred approximately once every 40 yr (in 1920, 1958, and 1995) in the Japan Sea (Shimomura 1959, Yasuda 2004, Uye 2011). In recent decades, however, especially between 2002 and 2016, *N. nomurai* has bloomed nearly every year at various levels (except 2008, 2010, 2011, 2014, and 2015) in the northern East China, Yellow, Korean and Japan seas (Ding & Cheng 2005, 2007, Zhang et al. 2012, Uye 2014, Yoon et al. 2014, S. Sun et al. 2015). Large outbreaks of medusae occurred from 2003 to 2007 and again in 2009. The blooms in 2012, 2013, and 2016 were of a moderate intensity.

Stinging and mortalities caused by *N. nomurai* have increased around the coastal seas of China in recent years, especially involving children and young teenagers (Dong et al. 2010). In 2005, the Fisheries Agency of Japan received thousands of complaints from fishermen because of clogged and bursting nets, reduced fish harvests, stung and dead fish, additional work to collect the fish and dispose of the medusae, and the risks of stings (Kawahara et al. 2006, Uye 2008, 2014). According to statistics, immediate financial losses to the fishing industry reached \$68.2 to 204.6 million annually in Korea (Kim et al. 2012). In addition to the threats to human society, there is a risk that the traditionally fish-dominated trophic structure might be destroyed, accompanied by the eventual dominance of jellyfish (Uye 2011). Many hypotheses on the possible factors contributing to jellyfish blooms have been proposed, including anthropogenic disturbances such as eutrophication, overfishing, aquaculture, habitat modification, and climate change (Purcell et al. 2007, Richardson et al. 2009, Purcell 2012, Uye 2014).

The benthic polyp stages of the *N. nomurai* life cycle (Fig. 1) are thought to determine the abundance of pelagic medusae through an autologous reproduction strategy (Kawahara et al. 2006, 2013, Uye 2014, authors' pers. obs.). Strobilation in spring and early summer results in the successful transformation of polyps to medusae by liberating multiple ephyrae (Kawahara et al. 2006, Dong et al. 2012, Feng et al. 2015a,b). Beforehand, the initial colonization of polyps is principally derived from spacious sexual propagation owing to the huge fecundity of medusae ( $\sim 10^8$  eggs for a developed female; Ohtsu et al. 2007, Ikeda et al. 2011), in addition to the preceding self-podocyst reproduction (Kawahara et al. 2006, Uye 2014). From August to October, massive planulae of *N. nomurai* are produced via external fertilization; these settle and metamorphose into early-developed polyps, which then further grow

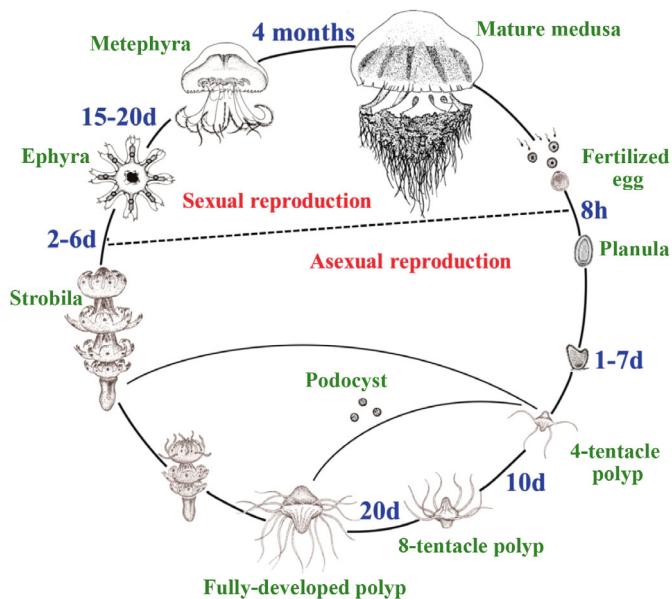


Fig. 1. Life cycle of *Nemopilema nomurai*. The formation of planulae, development of polyps and cultivation of ephyrae took place at 20°C in the laboratory. The metephyrae were cultured to the mature gonad of medusae at 20–23°C for 4 mo

and develop over the monsoon season (Ying et al. 2015, Gao & Xie 2016, Jia et al. 2016) across the northern East China, Yellow, and Bohai seas (Dong et al. 2012, Feng et al. 2015a,b, S. Sun et al. 2015), when decreased salinity may be a critical environmental pressure. During this stage, the early-developed polyps presumably would be more fragile and sensitive to environmental variations in contrast with fully developed polyps. Since the major birthplaces of *N. nomurai* medusae are located in the inshore estuaries (Kawahara et al. 2006, Toyokawa et al. 2012, Wang et al. 2013, Yoon et al. 2014, S. Sun et al. 2015), the hyposaline seawater in these regions likely contributes to the survival, development, and growth of the early-developed polyps and their subsequent asexual reproduction. Moreover, the biomass and production of micro-zooplankton, considered to be the main food for polyps (Ishii 2001, Kamiyama 2013), probably increases owing to the frequent recent occurrence of eutrophication in the estuaries (Wang et al. 2009, Uye 2011, Jiang et al. 2014). Here, their supply may also affect the growth and subsequent reproduction of the early polyps.

Therefore, in this study, the effects of hyposalinity and incremental micro-zooplankton supply were tested on 8-tentacled polyps cultivated in the laboratory at 11 salinity levels and 3 feeding frequencies at a constant temperature (19°C). Quantitative indices

on the survival percentages and full development of polyps, growth (calyx diameter), podocyst production, and excystment potential were continuously monitored for 100 d. The null hypotheses that survival, growth of early-developed polyps, and subsequent asexual reproduction did not significantly differ among salinities and food supplies were examined to further understand the mechanism of *N. nomurai* blooms.

## MATERIALS AND METHODS

### Polyp source

Six female and 4 male adult medusae of *Nemopilema nomurai* were captured by using a hand net in Laoshan Bay, Qingdao, China on 25 September 2013, and immediately transferred into a 30 m<sup>3</sup> (5 × 4 × 1.5 m) concrete pond at nearby Laodong Seafood farm. The pond water was renewed with filtered ambient seawater (smallest filter mesh: 0.5 mm; mean ± SD temperature and salinity: 20 ± 0.5°C and 30 ± 0.5, respectively) twice daily until they spawned on the third day. The medusae were then removed, and 100 polyethylene plates (40 × 30 × 0.5 mm) were introduced as substrates for planulae attachment. After 2 wk, when the majority of the planulae had developed into 8-tentacled polyps, the plates were transported to the laboratory at the Institute of Oceanology, Chinese Academy of Science.

### Laboratory experiments

The effects of hyposalinity and incremental micro-zooplankton supply on the physio-ecological properties of *N. nomurai* polyps were examined at a constant temperature (mean ± SD: 19 ± 0.5°C) for 100 d in the laboratory. The polyethylene plates were cut into small pieces (5 × 3 cm), so that one side of each piece bore 10 to 15 polyps. In total, 9 pieces were prepared for each of 11 different salinity treatments (8, 11, 13, 15, 18, 20, 23, 25, 28, 31, and 33). They were placed into a 10 l plastic container with membrane-filtered (0.45 µm) seawater at salinity 30, which was gradually diluted or concentrated to the desired salinity over 2 d, and acclimated at the respective salinity for 1 d. They were then individually transferred into 9 glass beakers, each containing 250 ml water; these were renewed with freshly-prepared water at 2 d intervals. Water of different salinities was made by diluting natural seawater (salinity: 30) with deionized

water or through the addition of coarse salt. Each salinity was checked with a CTD (AAQ1183-1F; Alec Electronics).

In each salinity treatment, 3 different feeding schedules were applied. A total of 50 cultured *Brachionus plicatilis* (mean ± SD body length: 191 ± 17 µm; carbon biomass: 0.16 mg C l<sup>-1</sup>), as a representative micro-zooplankton, were pipetted together with 1 ml of water (salinity: 30) to each beaker at 3 or 9 d intervals. An unfed schedule was also established. After polyps were fed randomly for 2 h, the water was replaced with a newly-prepared batch.

The numbers of polyps, podocysts, and excysted polyps were counted under a dissecting microscope (Nikon SMZ745) at 3 d intervals in the first month (from 7 October to 7 November 2013), and thereafter at weekly intervals. The diameters of poly calyx and podocysts were measured weekly with an eye-piece micrometer scale in the unfed case. If the calyx was not round, the mean of the maximum and minimum dimensions was taken as a diameter (Han & Uye 2010). After counting excysted polyps, the resulting polyps were removed with a needle.

### Calculations and statistics

For each salinity and feeding combination comprising 3 beakers, the following parameters were determined at 3 to 7 d intervals: polyp survivorship, composition of fully-developed polyps with ≥16 tentacles, comparative podocyst production potential (i.e. podocyst numbers divided by initial polyp numbers), comparative excystment potential (i.e. excystment incidences divided by total podocyst numbers), and the diameter of calyx and podocysts.

Repeated measures ANOVA was used to test for differences in the percentages of survival and full development of polyps, as well as calyx diameter and podocyst production among salinity and food supply treatments. The variance analysis of the podocyst diameter among salinity and food supply treatments was conducted via 2-way ANOVA. Percentages were arcsine square root transformed before statistical analysis. If the overall ANOVA results were significant, pair-wise comparisons were conducted with Fisher's least significant difference post hoc test. Data that failed to agree with the assumptions of normality or homogeneity of variance after transformation were analyzed with nonparametric analogues (Kruskal-Wallis 1-way ANOVA on ranks). A Tukey test or Dunn's method was used to analyze significant differences between treatments.

## RESULTS

### Polyp survivorship

During the acclimation period to the various salinities, the 8-tentacled polyps immediately retracted, then gradually re-extended and recovered at salinity treatments 11 to 23. However, those at salinity 8 remained retracted. The tentacles of polyps at salinity treatments 25 to 33 did not exhibit any response to salinity change. The survival percentage differed significantly among salinity and feeding combinations (Table 1). When fed,

over 80% of the polyps survived in salinities from 11 to 20 with no significant differences among combinations (Fisher's least significant difference), while the survival percentages at salinities 8, 28, 31, and 33 were ultimately <15% (Fig. 2). The number of polyps rapidly decreased in the high salinity treatments (28 to 33) during the development and growth of 8-tentacled polyps (during the first 23 d of the experiment) (Fig. 2). In low salinity treatments (11 to 20), survivorship decreased under starvation, but differences between feeding groups were not significant (Fisher's least significant difference).

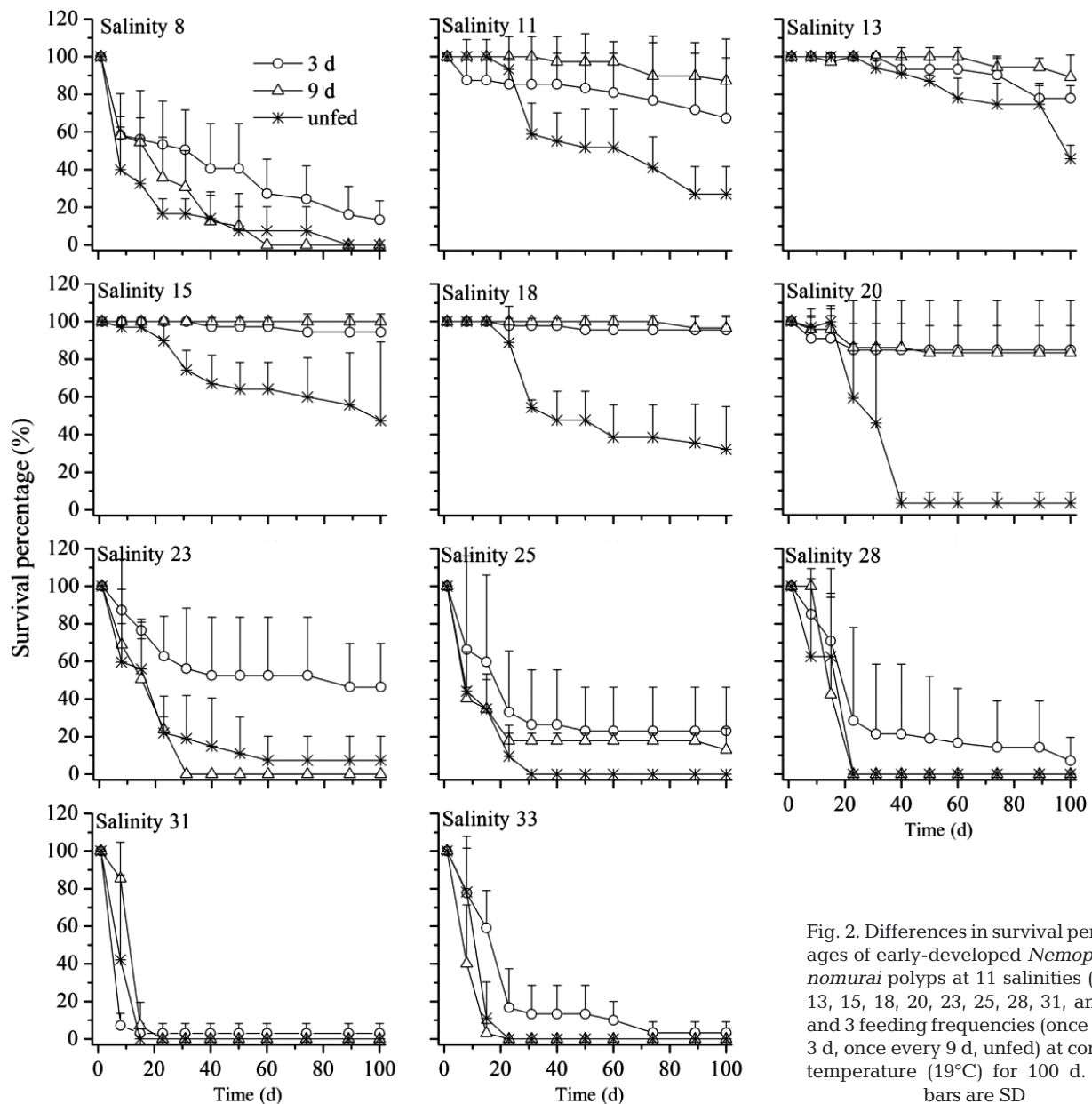


Fig. 2. Differences in survival percentages of early-developed *Nemopilema nomurai* polyps at 11 salinities (8, 11, 13, 15, 18, 20, 23, 25, 28, 31, and 33) and 3 feeding frequencies (once every 3 d, once every 9 d, unfed) at constant temperature (19°C) for 100 d. Error bars are SD

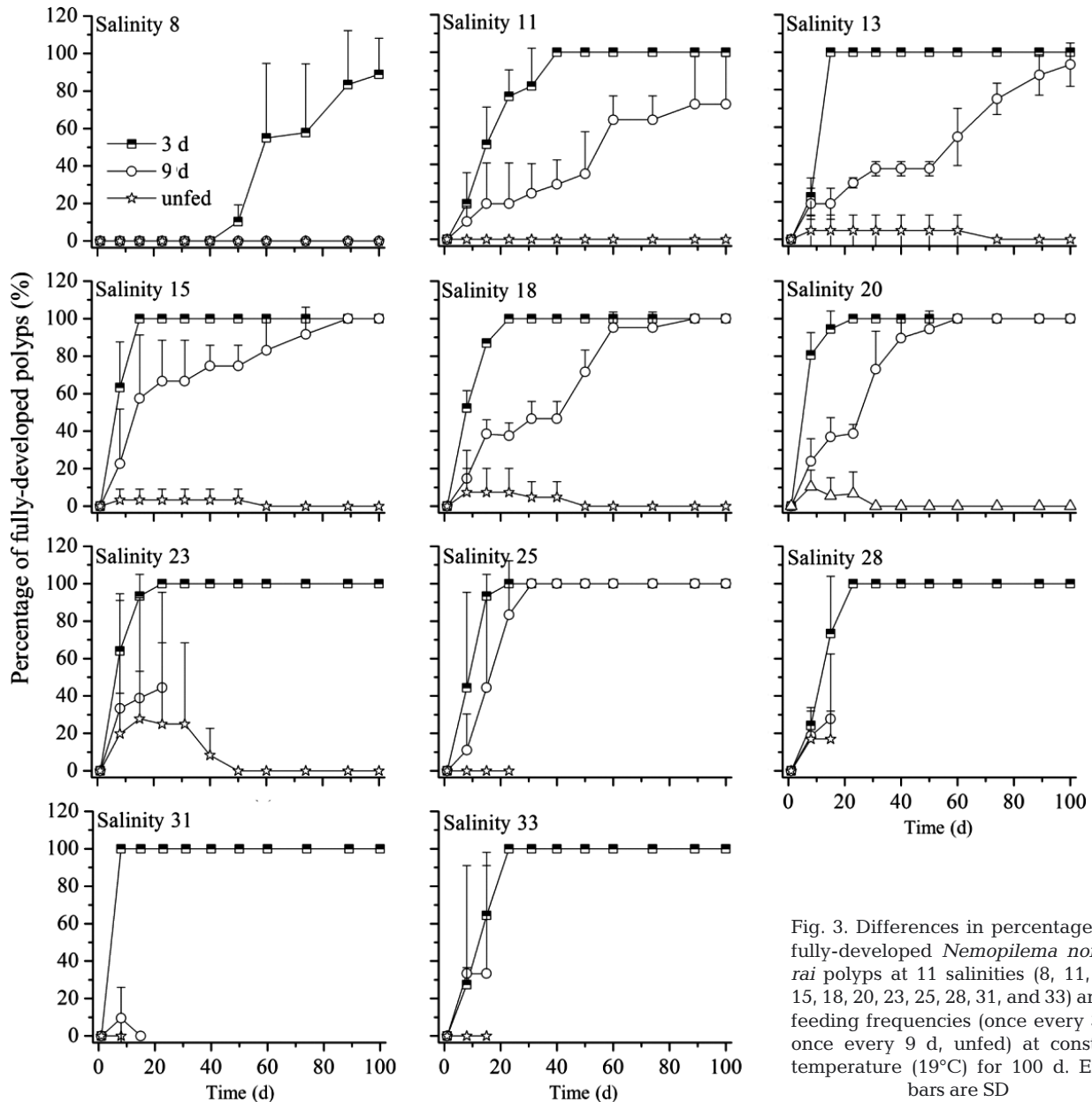


Fig. 3. Differences in percentages of fully-developed *Nemopilema nomurai* polyps at 11 salinities (8, 11, 13, 15, 18, 20, 23, 25, 28, 31, and 33) and 3 feeding frequencies (once every 3 d, once every 9 d, unfed) at constant temperature (19°C) for 100 d. Error bars are SD

### Development and growth

Fully-developed polyps with 16 tentacles appeared in all groups after 8 d except for salinity 8, and gradually accrued in the fed groups. However, they did not form in salinities 8, 11, 25, 31, or 33, or they first increased and later declined, with the number of tentacles reduced in salinities 13, 15, 18, 20, and 23 in the unfed group (Fig. 3). The percentages of fully-developed polyps were significantly influenced by food supply, but not salinity except at salinity 8 (Table 1). The numbers of 16-tentacled polyps were

greater when fed once every 3 d compared with once every 9 d from the beginning of the experiment.

During polyp development, calyx diameter increased in the fed groups, but remained relatively stable or slightly decreased in unfed groups (Fig. 4). The calyx diameter was significantly different among food supply treatments (Table 1), but not among salinities—again, except at salinity 8. The maximal calyx diameter was recorded at salinity 11 and in the frequently feeding group (every 3 d). Larger calyces were observed with more frequent feeding. The calyx diameter rapidly increased during the develop-



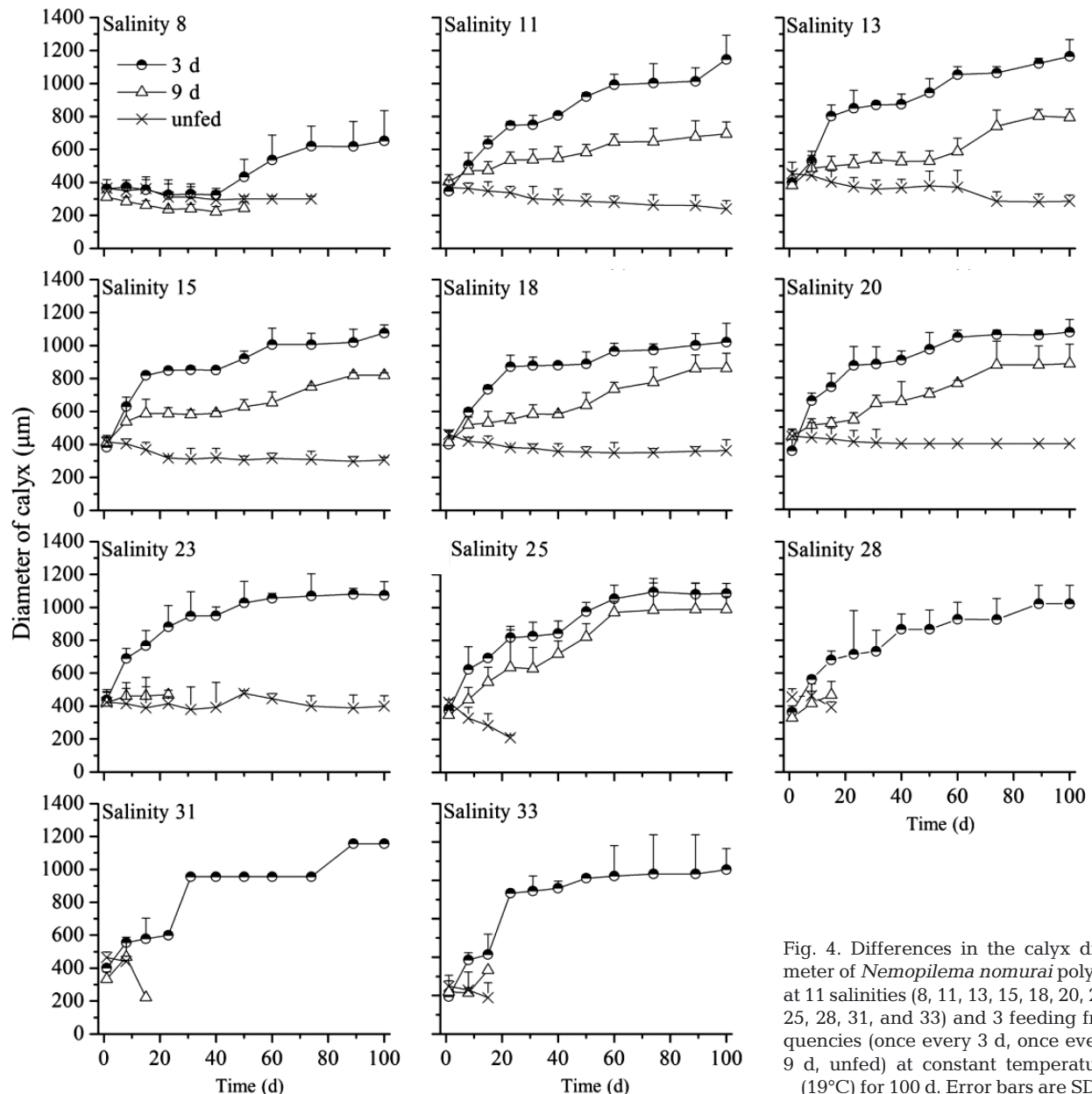


Fig. 4. Differences in the calyx diameter of *Nemopilema nomurai* polyps at 11 salinities (8, 11, 13, 15, 18, 20, 23, 25, 28, 31, and 33) and 3 feeding frequencies (once every 3 d, once every 9 d, unfed) at constant temperature (19°C) for 100 d. Error bars are SD

ment of the polyps from 8 to 16 tentacles in the frequently fed groups; the rate of increase was higher than in those fed once every 9 d. Polyps grew more slowly after development to 16 tentacles (Fig. 4).

#### Podocyst production and excystment potential

In the experiment, podocysts were only produced by polyps with 16 tentacles. The comparative podocyst production potential differed significantly among salinity and food supply combinations (Table 1).

Podocyst production did not occur at salinity 8. Podocyst production of polyp colonies was distinctly greater at middle salinities (15 to 23; Fig. 5). The maximum potential of podocyst production was recorded at salinity 20 and in the group fed once every 3 d. The numbers of podocysts increased dramatically from experimental Days 23 to 74 in the group fed once every 3 d, and afterwards remained relatively constant. However, in the feeding groups fed once every 9 d, podocyst number increased slowly along with increasing percentages of fully developed polyps (Fig. 5).

Table 1. Summary of repeated measures ANOVA results testing effects of salinity and food supply on survival percentage, percentage of fully-developed 16-tentacle polyps, diameter of calyx, and podocyst production potential of *Nemopilema nomurai*, as well as the summary of 2-way ANOVA on the diameter of the podocysts among food and salinity

Parameter	Treatment	— df —		F	p
		df1	df2		
Survival percentage of polyps	Salinity	10	88	32.050	<0.001
	Food supply	2	96	3.721	0.028
Percentage of fully-developed polyps	Salinity	9	50	0.856	0.570
	Food supply	2	69	78.715	<0.001
Diameter of calyx	Salinity	9	45	1.196	0.067
	Food supply	2	52	407.146	<0.001
Podocyst production	Salinity	10	82	3.611	0.001
	Food supply	2	90	25.789	<0.001
Diameter of podocysts	Salinity	9	41	1.806	0.096
	Food supply	2	48	14.333	<0.001

The diameter of podocysts differed significantly among food supplies (Table 1). Podocysts had larger diameters with more frequent feeding (Fig. 6). In contrast, salinity did not significantly affect the size of podocysts (Table 1). The excystment of podocysts was observed during the incubations, although not at salinities 8 or 11. The comparative excystment potential at salinities 23 to 33 was approximately 5 times higher than at lower salinities (13 to 20; Fig. 7).

## DISCUSSION

### Specific salinity adaptability in various development stages

On the basis of the experimental results, salinity affected the survival of early-developed 8-tentacled polyps of *Nemopilema nomurai* more than food supply. Early-developed polyps are more sensitive to salinity than fully developed polyps with 16 tentacles, over 90 % of which can survive at salinities from 10 to 30 (Dong et al. 2015). Early-developed polyps, however, only showed a high percentage of survival ( $\geq 80\%$ ) at salinities 11 to 20 in the present study. More than 80 % died during their development at high salinities (25 to 33). This also suggests that early-developed 8-tentacled polyps produced from sexual reproduction prefer a relatively low salinity environment.

ANOVA results regarding the composition of fully developed polyps and calyx diameter revealed that the development and growth of *N. nomurai* polyps were independent of salinity variation (except sal-

inity 8), suggesting potential euryhaline adaptability of early-developed polyps. Under high salinity stress, an average of approximately 10 % of early-developed polyps not only survived, but developed and grew, indicating their higher tolerance. After reaching 16 tentacles, polyps asexually produce podocysts in salinities from 10 to 33 (Dong et al. 2015, this study); however, high salinities (20 to 30) are more favorable for podocyst proliferation than low salinities (11 to 17) (Dong et al. 2015) at the individual level. In the present study, given the survival of the initially early-developed polyps, podocyst

production had the greatest potential for the preservation and self-proliferation of the original colony at salinity 20.

*N. nomurai* podocysts preferred to excyst at salinities from 23 to 33 rather than at those below 23. This is in agreement with the findings of Dong et al. (2015) that showed an optimum salinity for excystment ranging from 22.5 to 27.5. However, Kawahara et al. (2013) reported that more podocysts excysted at low salinity (from 8 to 27) in 80 d. The difference in these results may be explained by disparities in the podocyst experimental conditions. In the study by Kawahara et al. (2013), podocysts were placed into treatments of differing salinities directly from a salinity of 33; their results may have reflected the sudden change from high to low salinity. In our experiment, podocysts were produced in the same salinities in which they excysted, which eliminated the possible effect of an abrupt salinity change. To summarize, high salinity was detrimental to the survival of early-developed polyps formed from planulae, but stimulated subsequent podocyst production and excystment.

### *N. nomurai* polyps benefit from the potential increase in micro-zooplankton

Since polyps cannot actively move, accessible prey items may lie in high-density plankton, the supply of which *in situ* is therefore a crucial factor for the development and asexual reproduction of polyps (Spangenberg 1967, 1969, Han & Uye 2010, Wang & Li 2015). In the asexual generation of *N. nomurai*, the role of food conditions is seasonal in accordance with

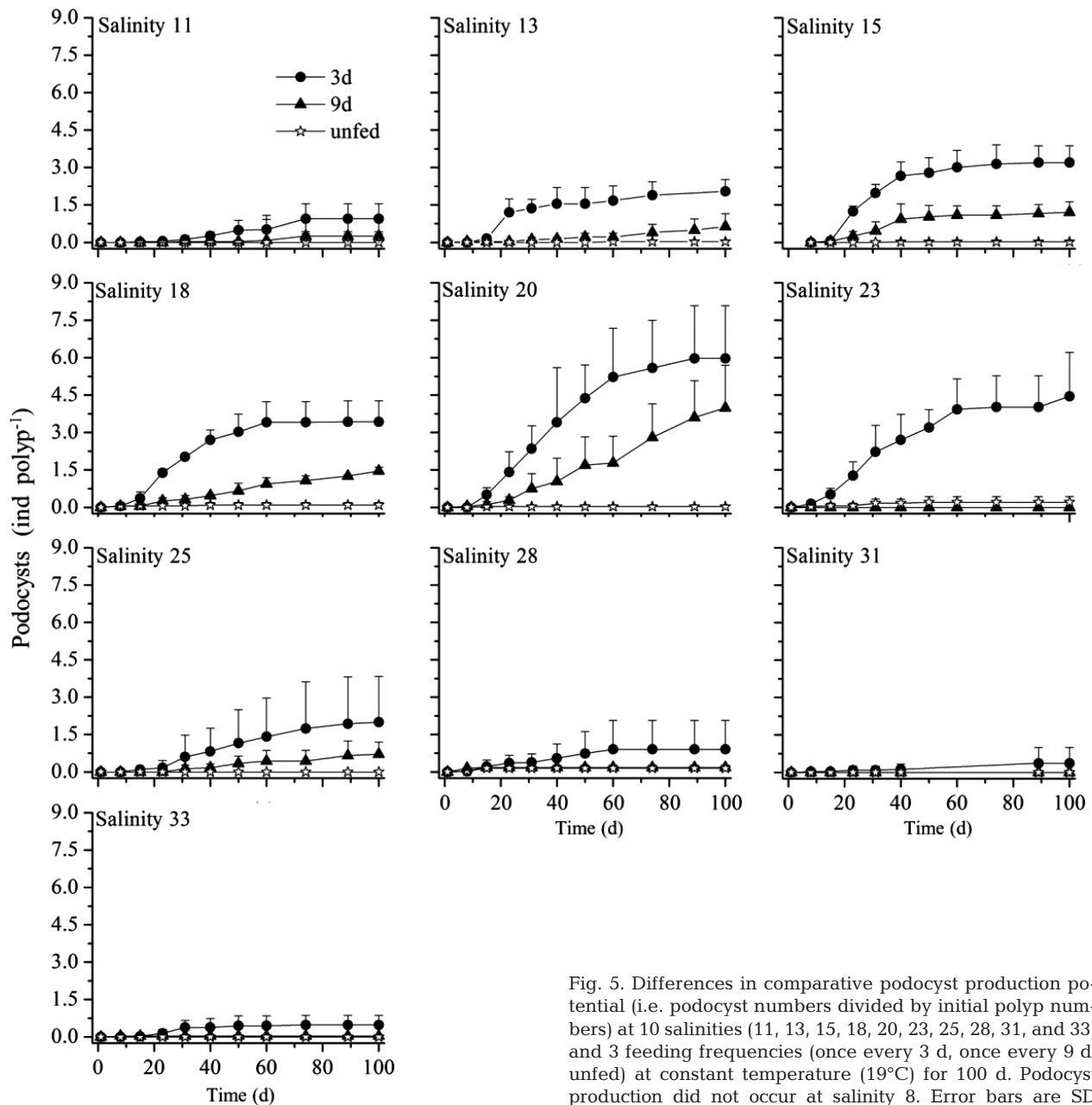


Fig. 5. Differences in comparative podocyst production potential (i.e. podocyst numbers divided by initial polyp numbers) at 10 salinities (11, 13, 15, 18, 20, 23, 25, 28, 31, and 33) and 3 feeding frequencies (once every 3 d, once every 9 d, unfed) at constant temperature (19°C) for 100 d. Podocyst production did not occur at salinity 8. Error bars are SD

temperature variation around the coast of China. In winter, polyps with little food availability cease growth and asexual reproduction due to extremely low temperature (M. Sun et al. 2015). After over-wintering for 2 or 3 mo, the fully-developed polyps appear to be more sensitive to the increase in temperature than the abundant food supply in spring (Feng et al. 2015a). Strobilae quickly develop with few prey (Feng et al. 2015a). Other undeveloped polyps probably first grow to reach sufficient energy reserves, and then start to strobilate (Feng et al. 2015a, M. Sun et al. 2015). After strobilation, podocysts are pro-

duced by surviving polyps and excyst at high temperatures in summer, when plankton supply significantly influences podocyst production (Feng et al. 2015b, M. Sun et al. 2015).

Apart from podocyst reproduction, generative propagation also acts as a prevailing recruitment for *N. nomurai* polyp population (Ohtsu et al. 2007, Ikeda et al. 2011). From August to October, polyps of *N. nomurai* are sexually produced in the northern East China, Yellow, and Bohai seas (Dong et al. 2012, Feng et al. 2015a, S. Sun et al. 2015). Based on the current findings, the ambient plankton supply in late



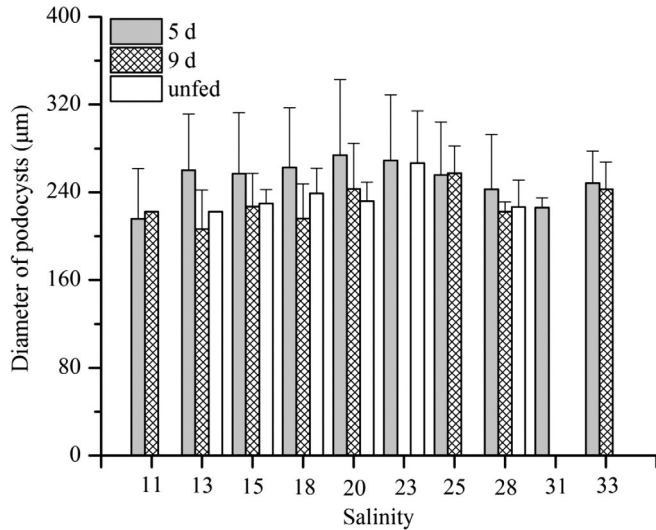


Fig. 6. Mean diameters of *Nemopilema nomurai* podocysts produced by polyps at 10 salinities (11, 13, 15, 18, 20, 23, 25, 28, 31, and 33) and 3 feeding frequencies (once every 3 d, once every 9 d, unfed) at constant temperature (19°C) for 100 d. Error bars are SD

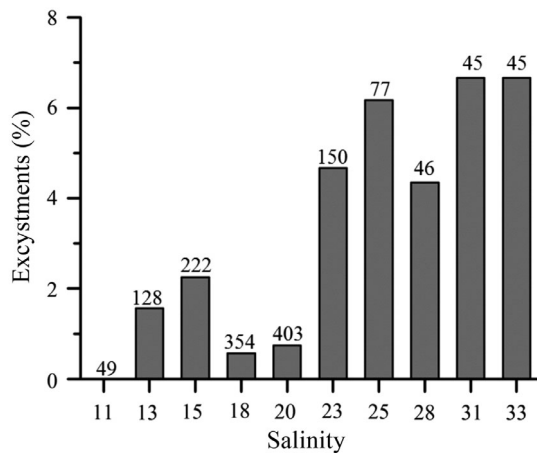


Fig. 7. Comparative excystment potential (i.e. excystment incidences divided by total podocyst numbers) of *Nemopilema nomurai* at 10 salinities (11, 13, 15, 18, 20, 23, 25, 28, 31, 33) and 3 feeding frequencies (once every 3 d, once every 9 d, unfed) at constant temperature (19°C) for 100 d. Numbers above the bars represent the total number of podocysts used in the experiment

summer and autumn directly influences the development and growth of early-developed polyps, as well as subsequent podocyst production. Since individual growth stops at low temperatures in winter and during strobilation in spring (Feng et al. 2015a,b, M. Sun et al. 2015), the size of fully-developed polyps would have been determined by the plankton supply in autumn. The number of ephyrae liberated is related to polyp size (Russell 1970). Therefore, adequate

plankton supply in autumn could increase the outbreaks of medusae in the following summer.

Based on field investigations of ephyrae and young medusae (Kawahara et al. 2006, Toyokawa et al. 2012, Wang et al. 2013, S. Sun et al. 2015), estuaries are thought to be the uppermost nursery grounds of *N. nomurai* medusae in east Asian marginal seas, including Changjiang and Liaodong Bay estuaries. As eutrophication has increased there recently (Wang et al. 2009, Jiang et al. 2014, Stokal et al. 2014), it is anticipated that the biomass and production of zooplankton may increase; however, community composition may shift towards miniaturization (Purcell et al. 2007, Uye 2011). These experimental results indicate that abundant micro-zooplankton supply could provide sufficient energy support for the development of *N. nomurai* polyps. Final calyx diameter and podocyst production of polyps fed with *Brachionus plicatilis* was not significantly different from those on a diet of *Artemia* nauplii (Feng et al. 2015b, M. Sun et al. 2015). From the vantage point of the pyramid principle of ecology, the density of micro-zooplankton in seawater is typically higher than that of meso- or macro-zooplankton, suggesting more frequent encounters with non-motile polyps, and therefore easier digestion (Kamiyama 2011). Size reduction of zooplankton might be conducive to assimilation by polyps, which is quickly transferred to asexual reproduction (Kamiyama 2013). Thus, *N. nomurai* polyps may benefit from the increase in micro-zooplankton.

#### Numbers of *N. nomurai* polyps were related to salinity

To the best of our knowledge, the discovery of *N. nomurai* polyp populations in the natural environment has not yet been published. The method of searching for the early ephyrae could help trace the birthplace of medusae. Regarding adaptability of early-developed polyps to the environment, the present results also provide strong evidence that the inshore hyposaline estuaries were the main home of *N. nomurai*, where regions of salinity from 10 to 20, as optimum habitat, were beneficial for the survival of early-developed polyps. While sexual reproduction occurred in late summer and autumn, the range of low salinities (10 to 20) at the bottom of nearby estuaries may determine the bloom intensity of medusae in the following summer.

To evaluate the possible role of salinity on *N. nomurai* outbreaks, the present study compared salinities in

the bottom waters of the Changjiang estuary in August when sexual reproduction occurs (Feng et al. 2015a, S. Sun et al. 2015). The areas of the previous years' low salinity (10 to 20) in non-blooming years (2008 and 2010) were smaller than those in the blooming year (2005) (Fig. 8). This indicates that the strength of Changjiang diluted water and monsoonal rainfall in August may regulate the abundance of *N. nomurai* medusae the following summer.

In addition to temperature induction as a major regulating factor (Feng et al. 2015a,b, M. Sun et al. 2015, Kawahara et al. 2013), the asexual reproduction strategy of *N. nomurai* is also matched with salinity fluctuations around the inshore Changjiang estuary, driven by freshwater and rainfall in later summer and autumn. In August, abundant early-developed polyps from sexual production of medusae could be recruited into suitable low-salinity

(10 to 20) regions. After 1 mo, polyps are fully developed (Kawahara et al. 2006, Uye 2014, Feng et al. 2015a,b, this study). As the monsoonal rainfall and strength of diluted water gradually weakens, the area of low salinity (10 to 20) at the bottom shrinks to the west coast from August to November (Fig. 8). Therefore, salinity in regions with newly-recruited polyps increases, stimulating polyp encystment to produce podocysts and excystment before temperatures decline to 18°C. Eventually, these polyps become an additional but crucial source of strobilating polyps in the following spring.

## CONCLUSIONS

Hyposalinity (from 11 to 20) significantly improved the survival of early-developed polyps; however,

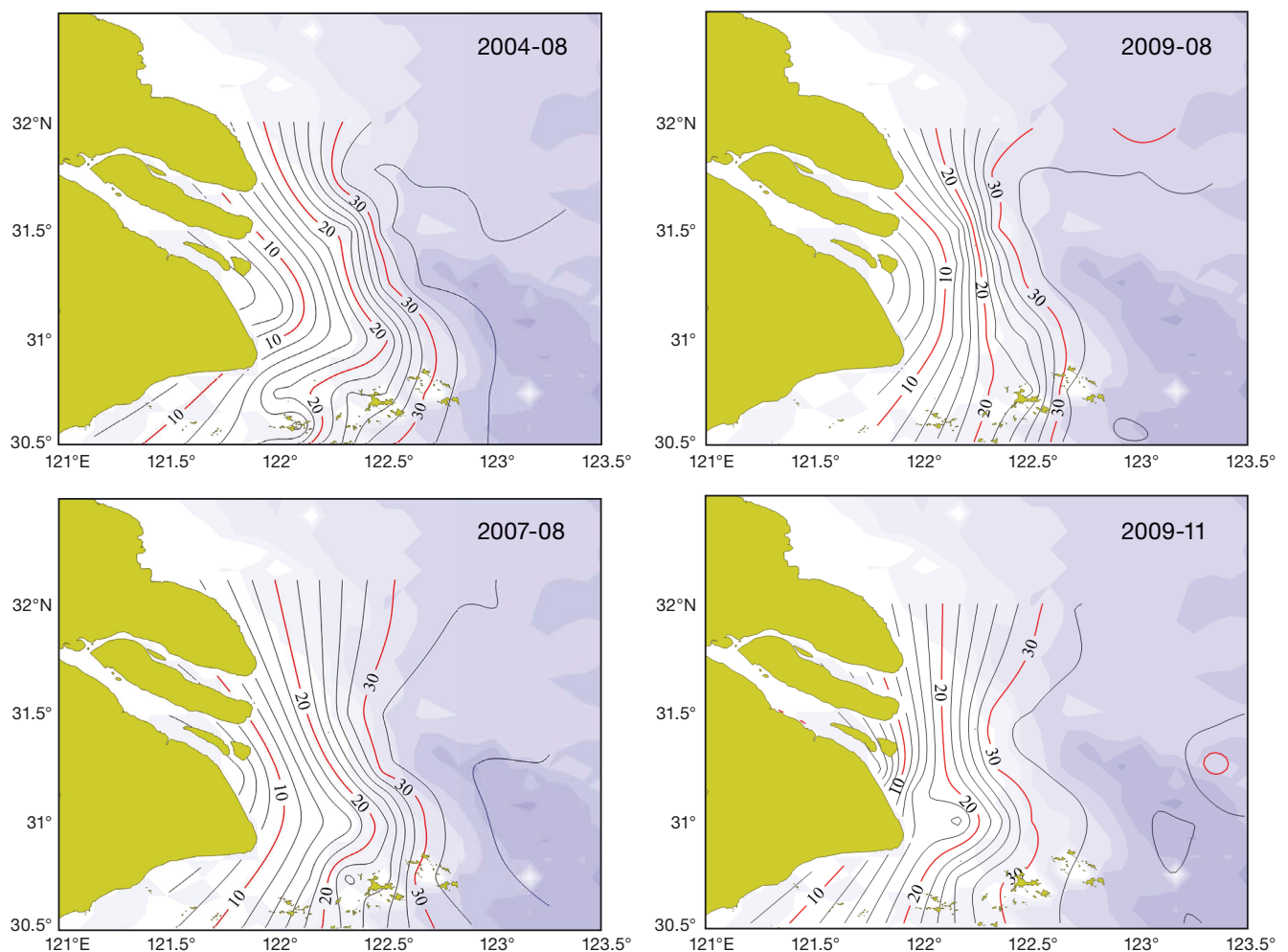


Fig. 8. Variation in regions of low salinity (10 to 20) at bottom around the Changjiang estuary inshore in August 2004, 2007, and 2009 and November 2009. *Nemopilema nomurai* medusae did not bloom in 2008 or 2010 when salinities during each preceding autumn were high; they did bloom in 2010, when salinity the preceding autumn was low

their development and growth were primarily controlled by micro-zooplankton supply in a positive way. The subsequent encystment of polyps was optimal at salinity 20 with an abundant food supply. Excystment was notably suppressed at hyposalinity <20. These results suggest that *N. nomurai* polyps probably extensively inhabit regions of low salinity (10 to 20) around the estuaries, where the growth and propagation process of polyps may benefit from the prospective increase in micro-zooplankton caused by the recent continual eutrophication. Meanwhile, the asexual reproduction strategy of these polyps is exactly adapted to salinity fluctuations in autumn. We conclude that not only plankton supply, but also the running freshwater and rainfall in autumn, may determine the medusa blooms the following summer.

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