DOCTORAL THESIS

A Comparative Study of Asexual Reproduction in Three Jellyfish, *Aurelia aurita* s.l., *Chrysaora melanaster* and *Cyanea nozakii* (Cnidaria: Scyphozoa: Semaeostomae), with Special Reference to the Role of Podocysts

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Abstract

The large gelatinous zooplankton such as cnidarian medusae and ctenophores have increased their abundance and caused problematic blooms in many coastal waters worldwide. In particular, in east Asian seas around Japan, China and Korea, where environmental conditions have been increasingly deteriorated by human impact, several cnidarian jellyfish species have become frequently blooming to cause severe damage to fisheries and other human economic sectors. In the light of increased jellyfish population outbreaks or blooms, it is necessary to understand the mechanisms to cause such phenomena. Because it is widely recognized that the asexual reproduction during benthic polyp stage may play a very important role to determine the medusa abundance in the following season, studies dealing with polyps have increased in recent years. However, few studies have been made on the role of podocysts, one of asexual reproduction modes of many semaeostome and rhizostome jellyfish species. The podocyts are a cuticle-covered-cell-mass formed by polyps and they are capable of dormancy until excystment into new active polyps. Therefore, in this study, I aimed to reveal the ecological role of podocysts in three semaeostome jellyfish species, viz. *Aurelia aurita* s.l.*, Chrysaora melanaster* and *Cyanea nozakii,* which are frequently blooming not only in Japanese coastal waters but also in Chinese and Korean waters, by conducting mainly laboratory experiments to determine the effects of different environmental factors (i.e. temperature, salinity, dissolved oxygen concentration and food supply) on their encystment, dormancy and excystment in addition to some histological studies.

In *A. aurita*, there are several modes of asexual reproduction by polyps, viz. budding and longitudinal fission, in addition to podocyst formation. The podocysts were never formed by well-fed polyps but only formed by un-fed and poorly-fed polyps (food regime: ≤4.8 μg C polyp^{-}

^{1} d^{-1}), and the podocyst production increased with the increase of temperature. The highest podocyst production during 8-week-experiment (i.e. 6 podocysts $polyp^{-1}$) was attained by un-fed polyp kept at 28^{o}C. The podocyst production was not affected by salinity within the range from 15 to 32. These results indicate that starvation is a trigger for encystment, while increased temperatures accelerate the encystment rate. The excystment was induced only when podocysts were exposed to cooling temperature (from 28 to 19 ^{o}C) and hypoxia (DO: 0.5 mg O_2 l^{-1}), indicating that the autumn is the main excystment season. The podocysts were capable of dormancy for up to 3.2 years, but old podocysts (i.e. 17-20 months old) excysted rarely (3%). A histological study revealed that newly-formed podocysts contained rich organic reserves (e.g. carbohydrates, proteins and lipids), and the initial reserves may have consumed during dormant period. Only a few nuclei and very weak reaction by RNA were found in the dormant cells of podocysts, indicating that the basal metabolism of the podocysts is low.

In *Chr. melanaster*, the podocyst production is an exclusive form of asexual reproduction by polyps. The production increased with the increase of temperature from 11 to 28 ^{o}C. It was lowest by un-fed polyps, and increased with the increase of food supply. A polyp, which was placed in the highest food supply (16.9 µg C $polyp^{-1}$) at 28^{o}C for 8 weeks, attained the highest production (16.5 podocysts $polyp^{-1}$). Salinity did not affect the podocyst production within the normal range (15-32) where polyps may encounter in the field. The excystment was high (33-48%) when the podocysts were exposed to decreasing temperature (from 28 to 11 ^{o}C), but only substantial (6-11%) when they were kept at constant temperatures (18 or 28 ^{o}C) and were exposed to increasing temperature (from 18 to 22 or 28 ^{o}C). These suggest that the podocysts of this species are capable of excystment without any specific temperature stimuli but attainment higher excystment under cooling temperature condition. Hence, their excystment may mainly

occur in autumn. Nearly 100% of podocysts excysted within 12 months even at constant temperatures, indicating that the maximal dormant period is ca. a year.

In *Cya. nozakii*, the podocyst production was also the only means of asexual reproduction and the previously-reported planulocyst formation was not observed. The podocyst production was significantly affected by temperature as well as food supply, both in positive manner, but the production tended to be saturated at temperatures ≥22 ^{o}C and at food supply levels ≥4.8 μg C polyp^{-1} d^{-1}. The highest podocyst production (6 podocysts polyp^{-1}) was attained by a polyp fed with 12.1 and 16.9 μg C polyp^{-1} d^{-1} at 26 and 28 ^{o}C, respectively. Within the salinity range from 15 to 32, the podocyst production was not affected by salinity. The excystment did not occur at all in the podocysts kept at constant temperatures (18 or 28 ^{o}C) and at increasing temperature (from 18 to 22 or 28 ^{o}C), but was induced highly (53-65%) when the podocysts were exposed to decreasing temperature (from 28 to 11 ^{o}C), indicating that the major excystment period of *Cya. nozakii* is autumn.

The present study has clearly demonstrated that the podocysts play important roles in the seasonal population dynamics of these three semaeostome jellyfish species. The major ecological roles of podocysts may lie into two aspects; one is for a reproduction to increase the polyp population abundance and another is for a refuge to protect the population from unfavorable environmental conditions. As the podocyst production is an exclusive form of asexual reproduction in *Chr. melanaster* and *Cya. nozakii*, the above-mentioned two aspects are equally important in their seasonal population dynamics. However, in *A. aurita,* the podocyts are produced only by starved and scarcely-fed polyps; they may play an important role as a strategy for population refuge to overcome unfavorable environmental conditions. In this species, budding is a common mode of asexual reproduction and the rate of budding is much higher than

that of the podocyst production. The type of strobila may also affect the increase of the medusa population abundance; it is poly-disc in *A. aurita* and *Chr. melanaster*, and mono-disc in *Cya. nozakii.* Hence, based on the above-mentioned modes and rates of asexual reproduction by polyps, the potential to increase the medusa population abundance, or to cause jellyfish bloom, is highest in *A. aurita*, followed by *Chr. melanaster* and *Cya. nozakii*. Since these basic biological features specific to each jellyfish species are influenced by environmental conditions (e.g. temperature, food supply and hypoxia), concomitant studies both in the controlled laboratory experiments and in field survey on both environmental variables and polyp and medusa population dynamics are always necessary in order to understand the mechanisms to cause jellyfish blooms.

Contents

Chapter 1. General introduction

Global expansion of problematic jellyfish blooms

A considerable amount of evidence has been accumulated to indicate that gelatinous zooplankton such as cnidarians and ctenophores have increased recently in many parts of the world ocean, and problematic jellyfish blooms have been expanding globally (Fig. 1). In east Asian seas around Japan, China and Korea, where the human-induced environmental and ecosystem changes are so marked as to be often called this area as an environmental hot-spot, many papers on jellyfish blooms and resulting problems have been published in recent years. For example, in Chinese waters, there are three problematic jellyfish species, e.g. *Aurelia aurita* s.l. Linneaus*, Cyanea nozakii* Kishinouye and *Nemopilema nomurai* Kishinouye*,* medusae of which have occurred in so large numbers as to damage not only in fisheries but also in other economic activities such as coastal power station operation and tourism (Cheng et al., 2005; Dong et al., 2010). In Korean waters, the shut down or power reduction of coastal nuclear power plants have more frequently taken place by aggregated *A. aurita* medusae, and at the same time the nuisance to fisheries has become increasingly significant (Lee et al., 2006). In many Korean eutrophic bays and inlets, such as Masan Bay, Jinhae Bay, Saemangeum, Shihwa Lake, etc. enormous numbers of *A. aurita* medusae have annually occurred showing conspicuous aggregations (C-H. Han, personal comm.).

In Japanese waters, more detailed information about the temporal shift of jellyfish occurrence is available. It was in the 1960s, when *A. aurita* population outbreak took place rather abruptly in Tokyo Bay, and the aggregated medusae clogged the intakes of cooling water of coastal power plants that resulted into blackouts in Tokyo metropolitan area (Sato, 1967;

Kuwabara et al., 1969; Matsueda, 1969). The *A. aurita* has since become one of the most dominant species in the zooplankton community components in Tokyo Bay (Omori et al., 1995; Toyokawa et al., 2000; Ishii & Tanaka, 2001). Since the 1980s, the *A. aurita* population significantly increased in the Inland Sea of Japan (Seto Inland Sea), according to the report based on an extensive poll to more than 1100 fishermen with professional experience over 20 years (Uye & Ueta, 2004). During the summer of 2000, a hitherto unobserved *A. aurita* bloom, the total wet-weight biomass of which was 9.4×10^4 tons, happened along the coastline in the Uwa Sea, western Shikoku (Uye et al., 2003). From the turn of this century, actually since 2002, the giant jellyfish *N. nomurai,* with maximum bell diameter and wet weight of ca. 2 m and 200 kg, respectively, has become frequently bloomed not only in Japanese waters but also in almost entire East Asian Marginal Seas (i.e. Bohai, Yellow, East China and Japan Seas). Because of large individual body size and massive occurrence of this species, fisheries, in particular net-fisheries, have been suffering severe damage and economical loss (Kawahara et al., 2006; Uye, 2008, 2011). In addition to remarkable increase of problematic blooms represented by *A. aurita* and *N. nomurai*, other scyphomedusae, such as *Chrysaora melanaster* Brandt and *Cya. nozakii*, have also hampered coastal fisheries, particularly in the Inland Sea of Japan (Ueda, 2007).

Apart from the east Asian waters, the Black Sea is one of the well-known waters with problematic jellyfish blooms. In the early 1980s, the ctenophore *Mnemiopsis leidyi* Agassiz was accidentally introduced perhaps by ship ballast waters from the east coast of the USA to the Black Sea, and then this species proliferated so abundantly as to destroy local fisheries (Kideys, 1994; Shiganova, 1998; Purcell et al., 2001). The flourished population invaded to the adjacent Azov Sea and finally to the Caspian Sea around 1990 (Ivanov et al., 2000). The distributional

rage of this species is currently expanding not only to entire Mediterranean Sea, but also to the North Sea and the Baltic Sea (Oliveira, 2007).

The northern Gulf of Mexico is a traditional shrimp fishery ground. Graham (2001) examined the records of jellyfish numbers caught by shrimp trawls since 1985, and found that *Chrysaora quinqueccirrha* Desor and *A. aurita* have been steadily increasing over the last decade or so. In addition, this area was infested by ca. 10 million medusae of *Phyllorhiza punctata* Lendenfelid, or Australian spotted jellyfish, with a mean bell diameter of ca. 45 cm, in the summer of 2000 along the coast of Louisiana and Alabama, USA. The unprecedented occurrence of this large jellyfish caused 10-million-USD loss to shrimping industry (Graham et al., 2001, 2003; Bolton & Graham, 2004).

The Namibian Benguela upwelling area is one of the world-most productive ecosystems, sustaining large fish stocks, such as sardines, anchovies and jack mackerels. However, heavy fishing pressure primarily by European fishing vessels has reduced fish stocks, and instead *Chrysaora hysoscella* Linnaeus and *Aequorea forskalea* Forsskål have increased as to attain total wet weight biomass of 12.2 million tons in the summer of 2003 (Lynam et al., 2006).

Lastly, I have to mention the most reliable long-term jellyfish monitoring in the Bering Sea. The shallow continental shelf of the east Bering Sea is a nursery ground of Alaskan pollocks, and hence the trawl survey in order to assess their stock has been operated since 1975. Jellyfish are certainly a bi-catch, but the numbers and weight of jellyfish caught in a trawl net have been recorded up to now (Fig. 2). During the 1980s, the jellyfish biomass was low and stable, but since1990 it increased dramatically, ca. 10 times of the 1980s' average biomass, toward a prominent peak in 2000. However, since 2000, the jellyfish biomass has been declining. These temporal variations of jellyfish biomass are not related to any human impact because of remote

location of the Bering Sea from densely populated cities, but to associate to regional climate change or regime shift (Brodeur et al., 2008).

Although I will not go any further, there are plenty of reports on jellyfish bloom incidents in the other regions of the world. Recent increase of scientific publications as well as mass communication articles (e.g. newspapers, magazines and web sites) on the jellyfish may indicate the global expansion of jellyfish blooms and at the same time increased scientific attentions to hitherto under-studied jellyfish.

Causes for jellyfish blooms

Many authors have argued that the causes for recent jellyfish blooms may be primarily attributed to human-caused changes, such as climate change, overfishing, eutrophication, marine construction and species introductions, since these may provide benefits for jellyfish populations to increase (Shiganova, 1998; Arai, 2001; Mills 2001; Parsons & Lalli, 2002; Purcell, 2005; Purcell et al., 2007; Lynam et al., 2006; Attrill et al., 2007; Uye, 2008). However, it is not easy to identify which factors are really responsible, since the causes may be multiple. The recent increase of the jellyfish bloom in the east Asian seas may not be attributed to long-term climate change, which is important for the temporal variations in jellyfish biomass in the Bering Sea, but to recent elevation of human impact to the coastal marine ecosystem.

Because jellyfish and fish are competitors for food as well as predators each other, overfishing, or the removals of fish by fishery more than their natural recruitment, may leave food sources (typical food for both groups: copepods) for jellyfish and allow the populations to utilize them to increase their biomass (Mills, 2001, Uye, 2008). The mortality of jellyfish, particularly in young stage such as ephyrae and metephyrae, must be high by fish predation, but is alleviated by overfishing to allow them to leave more jellyfish in the sea (Uye, 2011).

Eutrophication, or enrichment of nutrients such as nitrogen and phosphorus, accelerates the phytoplankton productivity, which may also lead to the increase of zooplankton, a primary food for jellyfish. East Asian coastal waters have been eutrophicated significantly, particularly in Chinese coastal waters (Yan et al., 2004; Wang, 2006; Dong et al., 2010) by the rapid economic growth. The Chinese coastal waters may become an ideal place for jellyfish to obtain sufficient amount of food to sustain their growth and reproduction (Uye, 2011).

Marine infra-structures, such as reclamation and construction of harbors, waterfronts and floating piers, may create new substrates for polyps to settle and form their colonies (Miyake et al., 2002, Uye, 2008).

Recent busy marine transportations have increased the species introductions for many marine organisms including jellyfish. In fact, several species of jellyfish have been introduced accidentally to new habitats, where they have proliferated (Purcell et al., 2007). One of the typical examples is the introduction of *M. leidyi* into the Black Sea (Vinogradov et al., 1989; Harbison, 2001), from where this ctenophore further invaded to the Azov Sea, the Mediterranean Sea and Caspian Sea (Purcell et al., 2001c; Graham & Bayha, 2007).

Life cycle of scyphozoan jellyfish

In the east Asian seas, jellyfish species that cause problematic blooms belong to the class Scyphozoa or scyphozoans. The life history of scyphozoans basically consists of an alternation of asexual benthic polyp phase and sexual pelagic medusa phase (Gröndahl, 1988; Arai, 1997). One well-known exception is *Pelagia noctiluca* Forsskal, which has no sessile polyp stage, since the planula larvae of this species develop directly into ephyrae (Goy et al., 1989; Rottini-Sandrini & Avian, 1983).

In most scyphozoans, particularly in the orders of Rhizostomae and Semaeostomae, or rhizostomes and semeostomes, respectively, there are several forms of asexual reproduction, viz. budding, stolonic budding, fission and podocyst production (Fig. 3, Arai, 1997). The budding and stolonic budding are the most common forms, where new polyps are formed on the parent's stalk and at the end of extended stolons, respectively. In the fission, one polyp splits into two polyps. The podocyst production does not mean the immediate increase of new polyps, but the podocysts later undergo to form new active polyps by excystment. The podocysts are formed against the substrate by the pedal discs of polyps or at the end of extended stolon (Pitt, 2000; Arai, 2009). The excysted polyps from the podocysts subsequently increase polyp population size by budding when the environmental condition is favorable, and those polyps are also capable of producing both further podocysts and medusae by strobilation (Arai, 2009). Compared to the other asexual reproduction forms (i.e. budding, stolonic budding and fission), the podocyst production has been less studied.

Brief overview of previous podocyst studies

According to Chapman (1968), the dome-shaped podocysts were first described more than a century ago by Hyde (1894) for *A. aurita*, but not until 1907 did Herouard (1907) suggest that the podocysts of *Chrysaora hysoscell*a Linnaeus might be an important part of the scyphozoan life cycle. Preliminary studies on cytology, formation, and excystment of podocysts of *Chrysaora* sp. were conducted by Herouard (1911, 1912a, b) and Tchéou-Tai-Chuin (1930), in addition to the reports of podocyst production in other jellyfish species, e.g. *Rhizostoma pulmo* Macri (Paspaleff, 1938), *Cyanea capillata* Linnaeus (Verwey, 1960), *Chr. quinquecirrha* (Littleford, 1939; Cargo & Schultz, 1966), *Rhizostoma octopus* Macri (Kühl,1972), *Rhopilema esculenta* Kishinouye (Ding & Chen, 1981; Lu et al., 1997). Chapman (1968) and Blanquet

(1972b) revealed that the podocysts of *A. aurita* and *Chr. quinquecirrha* were covered with a chitin-protein complex cuticle tanned by phenolic substances and stored carbohydrate-protein-lipid reserves. Recently, Kawahara et al. (2006) found that the podocysts are an exclusive form of asexual reproduction in the giant jellyfish *N. nomurai*, and Ikeda et al. (in press) found that the major chemical contents of organic reserves, such as carbohydrates, proteins and lipids, in *N. nomurai* podocysts were basically the same as those reported for *A. aurita* podocysts by Chapman (1968). Furthermore, Black (1981) reported that *Chr. quinquecirrha* podocyts could survive at maximum for 2 years, and Ikeda et al. (in press) confirmed that the dormant *N. nomurai* podocysts were capable of surviving for at least 5 years.

The effects of environmental factors influencing podocyst formation and excystment have been examined very preliminarily in limited species. For example, in *Cya. capillata* from Chesapeake Bay, USA, and *Cyanea* sp. from Connecticut, USA, the podocyst formation occurred during temperature warming period and the excystment occurred during temperature cooling season (Cargo, 1974; Brewer & Feingold, 1991). Similarly, podocysts of *Rho. esculenta* were not formed at temperatures below 10 ^{o}C, but produced at higher rates with the increase of temperature from 15 to 30 ^{o}C. In contrast, the podocyst formation of *Chr. quinquecirrha* occurred when temperatures was cooling toward 2-4^{o}C (Cargo & Schultz, 1967) and the excystment occurred at warm temperatures ranging from 15 to 18^{o}C (Cargo, 1974). In *N. nomurai*, the podocyst production was accelerated with the increase of temperature, and the excystment occurred when the podocysts were exposed to abnormal conditions such as high temperature, low salinity and hypoxia (Kawahara et al., 2006; Ikeda et al., in press). These results indicate that seasonal temperature variations are important for podocyst encystment and excystment.

Ecological significance of podocysts

Any scyphozoan podocysts have hard-shelled structure encapsulating a cell mass containing nutritional reserves, and are capable of dormancy for some prolonged period, up to 5 years for *N. nomurai*, until they excyst to form new polyps. The roles of podocysts may lie in several ways in the life cycle and population maintenance of scyphozoan jellyfish. First, needless to say, they contribute to the increase of polyp population abundance. Second, they can work as refugees from unfavorable conditions to survive as polyps, such as abnormally low (and sometimes high as well) temperature, salinity, dissolved oxygen concentration and food supply (Cargo & Schultz, 1966, 1967; Brewer & Feingold, 1991; Arai, 2009), in addition from predation by natural enemies such as nudibranches (Thiel, 1962; Cargo & Schultz, 1967; Hernroth & Gröndahl, 1985b; Gröndahl & Hernroth, 1987). Therefore, podocyts may play very important roles in population dynamics of scyphozoan jellyfish.

Objectives of this study

In the light of increased jellyfish blooms in east Asian seas in recent years, it is widely recognized that the asexual reproduction of polyps is a key factor to determine the medusa population abundance, and recent environmental changes in this area (e.g. eutrophication, water temperature increase, and marine construction) may actually accelerate polyp reproductive potential (Yan et al., 2004, Wang, 2006, Uye, 2011). As stated above, podocysts may play very important roles in scyphozoan jellyfish population dynamics. However, few works have been carried out on encystment and excystment of podocysts (Arai, 2009), and the ecological roles of podocyts in seasonal life cycle and bloom formation of scyphozoan jellyfish have never been

evaluated. Hence, the objectives of my thesis works are to understand the ecological roles of podocysts in the population dynamics of bloom-forming jellyfish.

I selected three semaeostome jellyfish, viz. *A. aurita, Chr. melanaster* and *Cya. nozakii*, which are common and blooming not only in Japanese coastal waters but also in Chinese and Korean waters, and conducted the controlled laboratory experiments to examine the physio-ecological properties of their podocysts. I studied their general morphological and histological features, effects of different environmental factors (i.e., temperature, salinity, dissolved oxygen concentrations and food supply) on both encystment and excystment of podocysts, and compared the physio-ecological properties among species. Finally, I discussed the ecological roles of podocysts in the seasonal life cycle as well as the bloom formation of these jellyfish species.

Chapter 2. Asexual reproduction of the moon jellyfish *Aurelia aurita* s.l. with special reference to the role of podocysts

Introduction

In Japanese waters, as in other temperate coastal waters, the moon jellyfish *Aurelia aurita* s.l. is the most common scyphozoan species. The taxonomic status of the genus *Aurelia* has not been yet organized, due to absence of static morphological characteristics of this gelatinous animals and wide morphological variations even in a single species. Recently, Dawson & Jacobs (2001), Dawson & Martin (2001), Dawson (2003) and Ki et al. (2008) conducted molecular studies for the genus *Aurelia* collected from many parts of the world, and could distinguish 10 cryptic species (i.e. *Aurelia* sp. 1~10) in addition to hitherto described species, viz. *Aurelia aurita* s.s. Linnaeus, *Aurelia labiata* Linnaeus and *Aurelia limbata* Brandt. Among the latter three species, *A. aurita* s.s. is distributed only in the Atlantic Ocean, mainly in temperate European coastal waters, and *A. labiata* and *A. limbata* are distributed in temperate and boreal waters of the North Pacific. The moon jellyfish in Japanese waters, which was previously called as *A. aurita*, has been designated as *Aurelia* sp. 1 by Dawson (2003) and Ki et al. (2008). The specimens having very identical (99.6% similarity) genotypes were also found in Korean, Californian (USA) and Australian waters, indicating that *Aurelia* sp. 1 is widely distributed over temperate and sub-tropical waters in the Pacific Ocean. Until a new species name has been established for this *Aurelia* sp. 1, I remain referring to *A. aurita* s.l. throughout my study.

In recent decades, unprecedented blooms of *A. aurita* have occurred, primarily on a seasonal basis in many Japanese coastal waters, where ephyrae are released from benthic polyps in winter and spring, grow rapidly to matured medusae in summer, and die in fall, although this

general pattern varies regionally (Miyake et al., 1997, Uye et al., 2003; Uye & Ueta, 2004; Kinoshita et al., 2006). Yasuda (1968, 1970, 1971, 1975) reported that a considerable numbers of medusae occur during winter in Urazoko Bay, a small bay on the coast of the Japan Sea. Similar over-wintering medusae, although the numbers are relatively small, have also been found in Tokyo Bay (Omori et al., 1995) and the Inland Sea of Japan (Uye & Ueta, 2004). It was in Tokyo Bay, where this species occurred very abundantly in the 1960s, when the bay was heavily eutrophicated by increased industrial and civil sewage discharge. They frequently blocked the water intakes of thermal power plants that used seawater to cool the condenser system, and caused operational difficulties or further shutdown of the power plants (Sato, 1967; Kuwabara et al., 1969; Matsueda, 1969). The *A. aurita* has since become the most dominant species in zooplankton components in Tokyo Bay (Omori et al., 1995; Toyokawa et al., 2000; Ishii & Tanaka, 2001). Since the 1980s, the *A. aurita* population significantly increased in the Inland Sea of Japan due to increased seawater temperature, decreased zooplanktivorous fish stocks and increased polyp settling area by marine constructions (Uye & Ueta, 2004). During the summer of 2000, the largest occurrence of *A. aurita* bloom, the total wet-weight biomass of which was 9.4×10^4 tons, occurred along the coastline in the Uwa Sea, western Shikoku (Uye et al., 2003). The blooms have become more conspicuous in many other Japanese coastal waters, e.g. Ise and Mikawa Bays, Hakata Bay, Yatsushiro Bay, off Sanin District, etc. (researchers of neighboring Prefectural Fisheries Experimental Stations, personal comm.).

The life cycle of *A. aurita* is comprised of an alternation of a planktonic sexual medusa phase and a benthic asexual polyp; the later plays a key role in determining their population size for the following season. Previous studies have demonstrated that the mode, rate or timing of asexual reproduction of *A. aurita* polyps are affected by light (Custance, 1964; Liu et al., 2009),

temperature (Spangenberg, 1968; Coyne, 1973; Willcox et al., 2007; Liu et al., 2009; Han & Uye, 2010), salinity (Segerstrale, 1957; Willcox et al., 2007), food availability (Spangenberg, 1964b; Keen & Gong, 1989; Han & Uye, 2010), concentrations of iodine and various iodocompounds (Silverstone et al., 1977), and predator density (Hernroth & Gröndahl, 1985b). Podocysts are one of the asexual reproduction products of *A. aurita* polyps, but their role in jellyfish population dynamics has been investigated rarely (Arai, 2009).

Because of increased jellyfish blooms during recent years, the importance of examining the asexual reproductive biology of benthic polyps is widely recognized (Purcell et al., 1999; Ishii & Watanabe, 2003; Ishii et al., 2008; Purcell, 2007; Willcox et al., 2007; Liu et al., 2009). In the benthic stage, polyps can increase and form colony by asexual reproduction so that more ephyrae are released through strobilation. Therefore, it is essential to investigate the dynamics during the polyp stage in order to understand the causes of medusa outbreaks (e.g. Ishii & Watanabe, 2001, Willcox et al., 2007). Hence, I conducted laboratory experiments to examine the effects of various environmental factors on the encystment and excystment of *A. aurita* podocysts to understand their ecological role in the population dynamics of this bloom-forming jellyfish species. I also conducted histological studies to examine the internal structures of podocysts and the changes associated with age.

Materials and methods

Effects of various conditions on podocyst formation

Preparation of polyps

Polyps of *A. aurita* were grown from planulae from a single mature female medusa caught during August 2007 from the Inland Sea of Japan, where water temperature was 28 ^{o}C

and salinity was 32. The polyps then were maintained as a stock-culture at 22 ^{o}C in filtered seawater of salinity 32. A batch of newly hatched nauplii of *Artemia* sp. (Utah, USA) was given as food two times per week. On 9 October 2007, experimental polyps were removed gently from the wall of stock-culture containers using a thin metal blade and placed individually in wells of 6-well polystyrene culture plates, each containing 10 ml of filtered seawater of salinity 32. The plates were kept at 22 ^{o}C for one week in darkness to ensure attachment to the well bottom until the start of podocyst-formation experiments. Effects of 3 environmental parameters, specifically, temperature, food supply and salinity were examined. During the experiment, newly-budded polyps were excised with forceps (Han & Uye, 2010), and strobilated polyps were monitored. Throughout the experiments, polyps were maintained in dark incubators (Nihon-ika Co.) except during feeding (10 min per week), water change (5 min per 3 days), and observation (1-10 min per day) except the experiment of dissolved oxygen concentration.

Combination effect of temperature and food supply

One culture plate with 6 polyps was placed at each of 6 temperatures (i.e., 5, 11, 18, 22, 26, and 28 ^{o}C±0.3 ^{o}C) in well-aerated (≥5.0 mg O_2 l^{-1}), 32 salinity seawater. At each temperature, polyps were fed 5 food levels (i.e. 0, 1, 2, 5, and 7 *Artemia* nauplii polyp^{-1} d^{-1}, corresponding to carbon supply of 0, 2.4, 4.8, 12.1, and 16.9 μg C polyp^{-1} d^{-1}, respectively) by placing a pipette containing nauplii near a polyp's tentacles (Table 1). Thus, the experiment consisted of 30 combinations of temperature (6 levels) and food (5 levels); each experiment ran for 8 weeks. To examine the combinations of the temperature and food supply on podocyst formation, two-way analysis of variance (ANOVA) was used in variance of the data (SPSS 10.0). If the overall ANOVA results were significant, Tukey pair-wise comparisons were performed to test among experimental combinations.

Effect of salinity

Each plate with 6 polyps was placed in a container of well-aerated (≥5.0 mg O_2 l^{-1}) seawater having different salinities (i.e., 5, 10, 15, 20, 25, 30, and 32; Table 2). The temperature was kept at 22 ^{o}C, and the food regime was 2.4 μg C $polyp^{-1}$ d^{-1}. The numbers of podocysts produced in different treatments of a parameter were analyzed by one-way ANOVA. If the overall ANOVA results were significant, the means were compared using Tukey pair-wise comparisons.

Effects of various conditions on podocyst excystment

Preparation of podocysts

For this experiment, two different ages of podocysts were prepared, i.e., young (≤1 month old) and old (17-20 months old) ones. To induce formation of podocysts, polyps from the stock cultures were cultured in polystyrene dishes (92x92x18 mm) at either 28 or 18 ^{o}C, fed with excess *Artemia* nauplii daily for 2 weeks, and then starved for one month. Then, the parent polyps were removed from the dishes and the remaining podocysts were kept in 32 salinity seawater at their respective temperatures in darkness before the experiment.

Effect of temperature

Both young and old podocysts initially at 28 ^{o}C were immediately transferred to lower temperatures (i.e., 19 and 11 ^{o}C); those at 18 ^{o}C were transferred to higher temperatures (i.e., 22 and 28^{o}C) to examine their excystment for 12 weeks. As controls, the podocysts were maintained at their initial temperatures throughout the experiment. In addition, the podocysts that were initially at 28 ^{o}C were exposed to lower temperatures in a step-wise fashion (i.e., 25, 22, 19, and

13 ^{o}C, each for 3 weeks), which resembled the seasonal seawater temperature decline. In each treatment, 34-43 young podocysts and 76-96 old podocysts were used in three replicates.

Effect of dissolved oxygen concentration

Podocysts of ≤1 month old, which had been produced in well-aerated (≥5.0 mg O_2 l^{-1}), 32 salinity seawater at 22 ^{o}C, were subjected to low dissolved oxygen concentration (DO) (target DO: 0.5 mg O_2 l^{-1}) established in DO bottles (volume: ca. 100 ml). The target DO seawater was obtained by purging oxygen by bubbling of nitrogen gas in an 8-l glass bottle, and then the water was gently siphoned into each DOB bottle. In order to examine the effect of duration under low DO, the podocysts were placed in the DO bottles for 1, 3, 7 and 14 days and then returned to the aerated condition to examine excystment for 4 weeks (Table 3). As a control, the podocysts were kept at the initial aerated conditions throughout the experiment. In all treatments except control, DO was measured by a DO meter (LDO^{TM} HQ10, Hach Co.) before and after experiment. Thirty podocysts were used in each treatment in three replicates.

Podocyst viability

In order to examine the ability of unexcysted podocysts to transform into polyps, following the excystment experiments, the chitinous covering of about one-half of the unexcysted podocysts (i.e. 5-30 podocysts) was artificially removed with a dissecting needle (Chapman, 1968). In addition, the cuticle was removed artificially at irregular intervals (ca. 3-12 months) from podocysts (i.e. 5-20 podocysts) that were produced at 18, 22, and 28 ^{o}C in well-aerated (≥5.0 mg O_2 l^{-1}), 32 salinity seawater, and kept under their respective conditions for various periods (up to 3.7 years) and still appeared to be viable.

Histological studies of podocysts

Histochemistry

Histochemical examinations were made using 1-month-old podocysts. They were fixed in a solution of 4% formaldehyde buffered with 30 mM HEPES (pH 7.4) for 1.5-2 h at room temperature. Fixed specimens were dehydrated using an ethanol series (50, 70, 90, and 100%) and embedded in LR-White resin (London Resin Company) polymerized at 60 ^{o}C. Sections (1 μm thickness) were cut on an ultramicrotome (Leica EM UC6rt) using a glass knife and stained using Mayer's Haematoxylin/Eosin technique for general morphology, the periodic acid schiff (PAS) technique for demonstration of carbohydrates, the acid solochrome cyanine technique for basic proteins and nucleic acids, the alcian blue technique for acidic and neutral mucopolysacchrides, and the methyl green-pyronin (MGA) technique for DNA and RNA. To identify lipids, podocysts were fixed in 2.5% glutaraldehyde and 2% formaldehyde buffered with 30 mM HEPES and post-fixed in 1% OsO_4 in the buffer for 1 h. Fixed specimens were dehydrated with the ethanol series and propylene oxide and then embedded in Quetol 651 resin (Nissin EN, Japan) at 60 ^{o}C. Sliced sections were stained using the Sudan black B techniques (McGee Russell & Smale, 1963). As controls, the sections were soaked in 2% potassium hydrate-ethanol solution to remove resin and lipids (Wada et al., 1993) and were stained using the same methods.

Change of internal structural with age

When newly-produced podocysts were detached from the parent polyps at 22 ^{o}C and 32 salinity, their locations and birth dates were recorded. Podocysts of known ages that looked viable, judging from the color of the inner cell mass, were removed from the substrate using a fine dissecting needle. They then were fixed in a solution of 2.5% glutaraldehyde and 2%

formaldehyde buffered with 0.1 M phosphate containing 0.4 M NaCl (pH: 7.4) and stored overnight at room temperature. After the fixation, a small hole was opened with the tip of a needle on the side of the podocyst to ensure penetration of reagents. The fixed podocysts were rinsed with the buffer three times at 15-min intervals using a shaker. These specimens then were post-fixed in a solution of 1% OsO4 made up in 0.1 M PO_4 buffer at 0^{o}C for 1 h, dehydrated through a graded series of ethanol. Finally, the podocysts were embedded in Quetol 65 resin at 60 ^{o}C and stored overnight. Sliced sections were mounted on glass slides, stained with 0.5% toruidine blue, and examined under an Olympus BX5 light microscope. To validate the different histological examinations, at least 5 podocysts were used in each staining method.

Results

General pattern of podocyst formation

The podocysts of *A. aurita* were opaque, whitish or light-brown in color, and dome-shaped, with diameter and height varying widely from 200 to 700 μm and from 60 to 110 μm, respectively. There were two types of podocysts formed, even by a single polyp: those formed at the base of the polyp (Fig. 4A) and at the end of extended stolon (Fig. 4B). The first type accounted for 85% of the 320 podocysts observed. Because there were no obvious differences in their shapes and sizes, both types of podocysts were used together in the experiments.

Effects of various conditions on podocyst formation

Combination effect of temperature and food supply

At 5 ^{o}C, all polyps strobilated and no podocysts were formed. At 11 ^{o}C, 90% of polyps strobilated without producing podocysts, but remaining 10% of polyps did not strobilate but

produced only a single podocyst, giving the overall average production of 0.3 and 0.2 podocysts polyp^{-1} at zero food supply and 2.4 µg C polyp^{-1} d^{-1}, respectively. In general, the warmer temperature, the earlier podocysts formed and the more were formed (Fig. 6). It was also general that the lower the food supply, the more podocysts formed. Actually, the number of podocysts produced differed significantly with temperature ($F_{5, 150}$=147.230, p<0.001), food supply ($F_{4, 150}$=257.472, p<0.001), and their interaction ($F_{20, 150}$=30.183, p<0.001) (Table 1, Fig. 5). The highest podocyst production (i.e. 6.0 podocysts polyp^{-1}) was attained by starved (i.e. no food supply) polyps, which were kept at 28 ^{o}C. It was notable that no podocysts were formed by well-fed polyps with food regimes of 12.1 and 16.9 µg C polyp^{-1} d^{-1} irrespective of temperature. In a given experimental condition, the podocysts production was relatively constant over 8 weeks, yielding a linear relationship between cumulative numbers of podocysts and duration in all experiments (Fig. 6).

Effect of salinity

At salinity 5, all polyps died within 2 weeks. At salinity 10, polyps appeared to be less active, having less-extended tentacles, than polyps in other treatments and they produced no podocysts. At salinity 15, polyps produced an average of 2.3 podocysts. Maximum production (average: 3.3 podocysts polyp^{-1}) was attained at salinity 32. There was no significant effect of salinity on the encystment within the salinity range of 15 to 32 ($F_{4, 25}$=2.591, p=0.061; Table 2, Fig. 7) at 22 ^{o}C.

Morphology and histology of podocysts

Internal fine structure

One-month-old podocysts of *A. aurita* is a dome-shaped contained a mass of dormant cells of undefined shape enclosed in a heavy cuticle-like capsule and outline of the roof surface is usually convex (Fig. 8A). Each cell had a very small nucleus (ca. 0.5 μm diameter) and many granules of various sizes, and cnidoblasts occurred in the cell mass (Fig. 8B).

Chemical contents

The histochemical methods demonstrated that 1-month-old podocysts contained carbohydrates and proteins, because the cell masses were extensively stained purple with the PAS method and red with acid solochrome cyanin (Fig. 9A, B). Traces of mucopolysaccarides, which were stained blue by alcian blue, were contained specifically within the cnidoblasts (Fig. 9C). Although nuclei containing DNA were clearly positive with methylgreen, the reaction of pyronin to RNA was very weak (Fig. 9D). Pyronin-stained cnidoblasts were notable, but this reaction was probably false (Fig. 9D). Small granules stained black with Sudan black corresponded to lipids (Fig. 9E). Particles from the control treatment showed diminished staining of lipids. These lipid granules were distinct from those of carbohydrates and proteins.

Change of internal structure with age

In newly-formed podocysts, the cell mass filled the entire internal space (Fig. 10A). The upper dome cuticle (2-4 μm thick) was relatively smooth, but the bottom cuticle was thicker and often irregular in thickness. One month later, the cell mass shrank slightly to leave small gaps (Figs. 8A, 10B). The gaps gradually became larger with the age (Figs. 10C, D, E) and the innermost layer of the cuticle became denser and thicker. Twelve-month-old podocysts looked mostly hollow, with only approximately 50% of the inner cell mass remaining (Fig. 10F).

General pattern of podocyst excystment

Before the transfer from 28 to 19 ^{o}C, the 1-month-old podocysts were filled with whitish-colored cell mass (Fig. 11A). Approximately 1-2 weeks prior to excystment, the cell mass began to shrink to form a round shape and moved to the upper part of the podocyst near an emergence hole. Excystment was accomplished by the cell mass extruding through the hole (Fig. 11B). Three to 5 days after excystment, the cell mass developed into a polyp with 4 rudimentary tentacles (Fig. 11C) and simultaneously began feeding. After an additional 4-10 days, it had developed to a 12-16 tentacled polyp (Fig. 11D).

Effects of various conditions on podocyst excystment

Effect of temperature

The excystment of young podocysts (≤1 month old) produced at 28 ^{o}C occurred only after transfer to 19 ^{o}C, where 7% excysted within 4 weeks and a total of 39% within 8 weeks; thereafter, no further excystment occurred (Fig. 12). In contrast, only 3% of the old podocysts (17-20 months old) excysted with the same treatment (Fig. 12). Neither the young or old podocysts produced at 18 ^{o}C excysted when transferred to warmer temperatures (22 and 28 ^{o}C).

Results from the stepwise decrease in temperature starting at 28 ^{o}C indicated that 6% of young podocysts excysted after they were transferred to 25 ^{o}C and then to 22 ^{o}C. The percentage of excystment increased to 15% after they were subsequently transferred to 19 ^{o}C, but no further excystment occurred despite additional cooling to 13 ^{o}C (Fig. 13). A similar stepwise temperature decrease did not result in excystment of old podocysts.

Effect of dissolved oxygen concentration

Although the target DO was 0.5 mg O_2 l^{-1}, the actual initial DO ranged from 0.48 to 0.52 mg O_2 $l^{-1,}$, and the final DO increased slightly less than 5% of the initial concentration. No excystment occurred for podocysts kept in aerated conditions throughout the experiment (total duration: 6 weeks). The podocysts that had been exposed to low DO for only 1 day, 11.8% of them excysted when they were returned to an aerated condition. The average excystment increased with the increase of duration under hypoxia to 32.5% for those which had been exposed for 14 days, showing a significant effect of hypoxic duration on the podocyst excystment ($F_{4,10}$=11.948, p<0.007, Table 3, Fig. 14). All excystment occurred within one week after returning to well-aerated seawater.

Podocyst viability

Even after the excystment experiments, the color and density of cell mass from the unexcysted podocysts looked the same as those before the experiments. When their cuticle cover was removed, the exposed cell mass was induced to transform from a planula-like form into a polyp. Occasionally, the removal caused a breakage of the cell mass resulting in several pieces, each of which transformed into a small polyp. Such artificial transformations took place in 80-100% of the podocysts examined, demonstrating that most of them could survive during adverse conditions that included a low salinity of 15 for at least 12 weeks and hypoxia around 0.5 mg O_2 l^{-1} for at least 2 weeks.

When podocysts were kept for periods longer than a year, the cell mass became darker color and markedly shrank in some podocysts (Fig. 15). Some of them acquired bacterial and/or fungal infections, which resulted in becoming empty shells (Fig. 15). Our sporadic examinations

of their viability by artificial shell removal revealed that the maximum longevity of *A. aurita* podocysts was 3.2 years at 18 and 22^{o}C while, only 2.4 years survive at highest temperature of 28^{o}C respectively. All podocysts died within 3.7 years in all temperatures (Table 4).

Discussion

Among various modes of asexual reproduction of *A. aurita* polyps, budding and strobilation are the major means to increase population abundance. The rate of budding accelerates with increases of temperature or food supply (Ishii & Watanabe, 2003; Willcox et al., 2007; Liu et al., 2009; Han & Uye, 2010). Strobilation, by which a single polyp produces several ephyrae, is induced primarily by lowering temperature below a threshold (ca. 15 ^{o}C, Kakinuma, 1962; Arai, 1997; Lucas, 2001; Ishii & Watanabe, 2003). On the other hand, the podocyst production rate is much lower (maximum: 0.75 podocysts polyp^{-1} week^{-1}, Table 1) as compared to budding (maximum: 8.1 buds week^{-1}, Han & Uye, 2010), suggesting that podocysts do not increase polyp numbers.

In order to induce encystment of *A. aurita* polyps, both food supply and temperature were important environmental factors. My results agreed with those of Han & Uye (2010) in that podocysts were never formed by well-fed polyps but only by unfed and poorly-fed ones, suggesting that starvation was the primary cue to induce podocyst formation in *A. aurita*. Below some food threshold, which may exist between 4.8 and 12.1 μg C polyp^{-1} d^{-1} (Fig. 5), *A. aurita* polyps may shift the allocation of nutrition from bud formation to podocyst production. Podocysts were produced by starved polyps even at 11 ^{o}C, and their production rate accelerated with increasing temperature to 28^{o}C.

Several previous studies demonstrated the importance of temperature change (either increase or decrease), rather than food supply, on podocyst production. In *Rho. esculenta*, podocyst production increased with temperature increased from 15 to 30 ^{o}C (Lu et al., 1997). In *Cya. capillata* polyps from both the Chesapeake Bay and Niantic River estuary, Connecticut, podocyst formation occurred when the water temperature underwent seasonal warming (Cargo, 1974; Brewer & Feingold, 1991). In contrast, podocyst formation of *Chr. quinquecirrha* from the Chesapeake Bay increased when water temperatures cooled (Cargo & Schultz, 1967). Podocyst production was never induced by changes over wide ranges of salinity (15-32) usually encountered by polyps in east Asian coastal waters.

In order to induce excystment of *A. aurita* podocysts, both cooling and deoxygenation were effective environmental factors. It is interesting that the sudden cooling from 28 to 19 ^{o}C was more effective than the step-wise cooling. Temperature also was important for excystment of *Cya. capillata* podocysts from the Chesapeake Bay, when it occurred upon seasonal cooling (Cargo, 1974; Brewer & Feingold, 1991). In contrast, excystment of *Chr. quinquecirrha* podocysts from the Chesapeake Bay occurred when the water temperature rose (Cargo & Schultz, 1967; Cargo & Rabenold, 1980). The effect of low oxygen (i.e., $\leq$1.0 mg O_2 l^{-1}) was striking; all excystments occurred within one week after returning to well-aerated seawater. Because summer deoxygenation is common in eutrophic coastal waters, such as in Tokyo Bay, Japan (Ishii et al., 2008), Chinhea Bay, Korea (Lim et al., 2006), and the Bohai Sea, China (Dong et al., 2010), podocysts that are exposed to the hypoxia may excyst during fall when the water is re-aerated by vertical mixing.

Previous studies reported that some nudibranch species consumed polyps of *A. aurita, Chr. quinquecirrha*, and *Cyanea* spp., but they did not consume the podocysts (Thiel, 1962;

Cargo & Schultz, 1967; Hernroth & Gröndahl, 1985b; Gröndahl, 1988; Gröndahl & Hernroth, 1987). The information is also available on the predation of a nudibranch, *Hermissenda crassicornis,* a gastropod, *Calliostoma unicum* and a shrimp, *Rhynchocinetes uritai*, which can consume >300 *A. aurita* polyps d^{-1}, but they did not consume any podocysts (Uye el al., unpublished data). These facts suggest that podocyst production also is a life-cycle strategy to protect the polyps from predators.

The general morphology, internal structure and major chemical contents of organic reserves from *A. aurita* podocysts in our study were basically the same as those reported by Chapman (1968). Our study showed that staining of RNA from the organic-matter-rich, dormant cells was very weak, indicating that gene expression activity was low during encystment. Thus, basal metabolism of the dormant cells might be low (Black, 1981), enabling the podocyst to live longer. Our histological study revealed that approximately half of the initial reserves were consumed during one year of dormancy. Black (1981) reported a similar amount of reduction for *Chr. quinquecirrha* podocyst, in which half of the DNA, one-third of the proteins, and one-fifth of the lipids contained in newly-formed podocysts were lost during one year. In our study, the maximum longevity of *A. aurita* podocysts was 3.2 yr, similar to the duration of dormancy reported by Herouard (1911). Because the success of excystment from the 17-20-month-old podocysts in the laboratory was very low (3%), actual excystment of older podocysts might become diminished over time due to the decreases in their reserves.

In temperate east Asian coastal waters, where the annual water temperature ranges roughly from 5 to 30 ^{o}C, the occurrence of *A. aurita* medusae shows a remarkable seasonal pattern as schematically depicted in Fig. 16 (Yasuda, 1983; Omori et al., 1995; Uye & Shimauchi, 2005). Typically, they appear in the plankton as ephyrae during late winter and early

spring, grow to a bell diameter of ca. 20 cm and attain sexual maturity in early summer, when the population biomass reaches its annual peak. After mid-summer, the medusae become senescent and disappear rapidly from the plankton. Sexually-reproduced planula larvae attach to substrates and metamorphose to polyps, entering the benthic phase of the life cycle, which may be perennial. The polyps increase their numbers by asexual reproduction (i.e., budding) at high rates when food supply and water temperature are both high. As water temperature decreases, they reproduce at lower rates, and when it is ≤15 ^{o}C, they transform to strobilae. This study adds new ecological significance of podocysts in the seasonal life cycle of *A. aurita*. When polyps are exposed to low food conditions, they reduce or stop budding and instead produce podocysts. Summer is the major season for podocyst production because most were produced at the highest temperatures. In fall, induced by exposure to cooling or recovery from summer hypoxia, these podocysts can excyst to polyps, which maintain the benthic population by budding until them strobilate. Unexcysted podocysts remain dormant until further stimulated. Although their excystment success in the laboratory was very low, they could survive for at least three years.

In *A. aurita* polyp population dynamics, podocysts may contribute minimally to the immediate increase of the polyp abundance. Nevertheless, they can maintain the population during adverse environmental condition (e.g., hypoxia) and also predator attack. These endurance potentials allow podocysts to act as temporal population outposts in coastal areas where polyp survival was marginal. Once these podocysts are established in such areas, future blooms of *A. aurita* medusae could occur.

Chapter 3. Asexual reproduction of the Japanese sea nettle *Chrysaora melanaster* with special reference to the role of podocysts

Introduction

In recent years, the expanding influence of anthropogenic activities has caused significant changes in coastal marine ecosystem, as represented by harmful algal blooms (or red tides), dead zones (or hypoxia), and loss of biodiversity. In addition, it is currently argued that jellyfish blooms, which have become increasingly more extensive and frequent in a variety of coastal regions worldwide, might also be attributed to the human-induced perturbation (Parsons & Lalli, 2002; Uye & Ueta, 2004; Lynam et al., 2006; Purcell et al., 1999, 2007; Uye, 2008).

The jellyfish of the genus *Chrysaora,* generally called as sea nettle, consists of 8 species, among which *Chr. hysoscella* Linnaeus, *Chr. lactea* Eschscholtz, *Chr. plocamia* Lesson and *Chr. quinquecirrha* Desor have been reported to occur in the Atlantic coastal waters (Mianzan & Cornelius, 1999), and *Chr. achylos* Brandt, *Chr. fuscescens* Brandt, *Chr. melanaster* Brandt and *Chr. pacifica* Goette have been reported to occur primarily in the north Pacific waters (Kishinouye, 1902, 1910; Austin, 1985; Martin, 1997; Morandini & Marques, 2010; Toyokawa, 2011). The bloom-forming species of the genus *Chrysaora* are represented by *Chr. fuscescens*, *Chr. hysoscella*, *Chr. quinquecirrha* and *Chr. melanaster*. In Chesapeake Bay, USA, *Chr. quinquecirrha* is a predominant summertime scyphomedusan species (Cargo & Shultz, 1967). This jellyfish species is voracious consumers of zooplankton and ichthyoplankton to cause damage to estuarine fish populations (Purcell, 1992; Cowan & Houde, 1993; Purcell et al., 1994a, b), and in fact a food web analytical model has demonstrated that the high trophic positions and high abundance of this species play an extremely important role in the plankton dynamics during

summer in Chesapeake Bay (Baird & Ulanowicz, 1989). In the northern Benguela upwelling ecosystem off Namibia, *Chr. hysoscella* population has increased since the 1990s to make nuisance to fisheries and diamond mining industries (Lynam et al., 2006). In the northern California current off Oregon, USA, *Chr. fuscescens* has occurred abundantly with a maximum density of 77 medusae 1000 m^{-3}, which may cause significant influence to the food web structure by voracious predation pressure on zooplankton that are also important food for many pelagic fish species (Purcell & Sturdevant, 2001; Suchman & Brodeur, 2005). In the Bering Sea, the biomass of *Chr. melanaster* increased during the 1990s to form a peak in 2000, and thereafter it is declining (Brodeur et al., 2008). In Japanese coastal waters, *Chr. melanaster* is a common scyphomedusa, appearing mainly in spring and early summer, often together with *A. aurita* medusae, to make nuisance to fisheries (Uye & Ueta, 2004; Kinoshita & Hiromi, 2005; Kinoshita et al., 2006; Ueda, 2007).

According to previous studies (Cargo & Schultz, 1967; Cago, 1974; Cargo & Rabenold, 1980), the asexual reproduction by polyps of *Chrysaora* spp. is performed only by means of podocysts, from which new active polyp's excyst to increase their abundance. It has been demonstrated that in *Chr. quinquecirrha* from Chesapeake Bay, the podocysts are produced during autumn and winter when water temperature is cooling down toward 2-4^{o} C, and they excyst during spring and summer when temperature is rising toward 15-18^{o} C (Cargo & Schultz, 1967; Cago, 1974; Cargo & Rabenold, 1980). Blanquet (1972) has examined the morphological structure and chemical composition of *Chr. quinquecirrha* podocysts, and demonstrated that they are dome-shaped structure measuring 0.3-0.5 mm in diameter, encapsulating a mass of whitish tissue inside. The capsule is made of a brown-colored cuticle with thickness of 9-13 μm, which is arranged by a series of lamellae. Furthermore, Black (1981) has reported that the one-year-old

podocyts of *Chr. quinquecirrha* have lost a half of DNA, one-third of proteins, and one-fifth of lipids contained in newly-formed podocysts.

Strobilation, a transitional stage from polyp to medusa, of *Chr. quinquecirrha* from Chesapeake Bay took place when the temperature was increasing (Cargo & Schultz, 1966, 1967; Cargo & Rabenold, 1980; Cargo & King, 1990; Purcell et al., 1999, Condon et al., 2001). On the contradictory, in *Chr. melanaster* from Mutsu Bay, northern Japan, the strobilation occurred in October and November when the temperature was decreasing (Kakinuma, 1967). Recently, Toyokawa (2011) found that the strobilation of this species (he used the name of *Chr. pacifica* in place of *Chr. melanaster*, since he thought them as synonyms) took place when the polyps were placed at low temperatures ranging from 5 to 10 ^{o}C.

Compared to American sea nettle *Chr. quinquecirrha*, few have been studies on the biology and ecology of the polyp asexual reproduction in Japanese sea nettle *Chr. melanaster*. Toyokawa (2011) recently found for the first time the wild polyps and podocysts of this species on pebbles and bivalve shells from shallow sea-bottom of Sagami Bay, middle Japan, and conducted a preliminary study in regard to strobilation. Because of recently increased bloom incidents by this species in many parts of Japanese waters, it is of importance to examine the asexual reproduction during the benthic polyp stage in order to understand the mechanisms for medusa population outbreaks. For this reason, following the study with podocysts of *A. aurita*, I investigated the effects of various environmental factors (i.e., temperature, salinity, food supply, dissolved oxygen concentration) on encystment and excystment of the podocysts of *Chr. melanaster*.

Materials and methods

Effects of various conditions on podocyst formation

Preparation of polyps

Polyps of *Chr. melanaster* used in this experiment were derived from the stock culture population maintained in seawater of salinity 32 at 22 ^{o}C. The stock culture originated from mature medusae caught in March 2009 from the Kanmon Strait, Yamaguchi Prefecture, the western extremity of the Inland Sea of Japan. In order to obtain fertilized eggs and planulae by artificial fertilization, the gonadal fragments were cut off from the medusae on site, and transported to a laboratory at Hiroshima University. Small pieces of ovary and testis (ca. 10 g, respectively) from 3-5 specimens were placed together in plastic vessels containing filtered seawater of 32 salinity at 18 ^{o}C overnight in darkness. Then, fertilized eggs and swimming planulae were isolated with a pipette under a dissecting microscope and transferred to plastic vessels (diameter: 130 mm, depth: 55 mm) containing filtered seawater to make them settled on the bottom. These vessels were kept in the dark at 22 ^{o}C, and newly transformed polyps were fed with a batch of newly hatched nauplii of *Artemia* sp. (Utah, USA) at 2-3-day intervals to establish the stock cultured of polyps.

On 10 July 2009, ca. 3-month-old-polyps with average stalk diameter of 300 μm, which had not produced any podocysts yet, were removed gently from the wall of stock-culture containers using a thin metal blade and placed individually in wells of 6-well polystyrene culture plates, each containing 10 ml of filtered seawater of salinity 32. The plates were kept at 22^{o}C for a week in darkness to ensure attachment to the well bottom until the start of podocyst formation experiments. Following the experimental procedures used in *A. aurita,* I examined the combined

effects of temperature and food supply, and the effect of salinity on podocyst formation by *Chr. melanaster* polyps.

Combination effect of temperature and food supply

As shown in Table 1, the experiment consisted of total of 30 different combinations, each using 6 polyps, of temperatures (i.e., 5, 11, 18, 22, 26, and 28 ^{o}C±0.3 ^{o}C) and food supply (i.e. 0, 2.4, 4.8, 12.1, and 16.9 µg C polyp^{-1} d^{-1}) using well-aerated (≥5.0 mg O_2 l^{-1}), 32 salinity seawater. Each experiment ran for 8 weeks. To examine the effect of combinations of the temperature and food supply on podocyst formation, two-way analysis of variance (ANOVA) was used in variance of the data (SPSS 10.0). If the overall ANOVA results were significant, Tukey pair-wise comparisons were performed to test among experimental combinations.

Effect of salinity

As shown in Table 2, 6 *Chr. melanaster* polyps were placed in well-aerated (≥5.0 mg O_2 l^{-1}) seawater having different salinities (i.e., 5, 10, 15, 20, 25, 30 and 32). The temperature was kept at 22 ^{o}C, and the food regime was 2.4 µg C polyp^{-1} d^{-1} throughout the experiment. The numbers of podocysts produced in different salinities were analyzed by one-way ANOVA. If the overall ANOVA results were significant, the means were compared using Tukey pair-wise comparisons.

Effects of various conditions on podocyst excystment

Preparation of podocysts

For this experiment, only young (≤1 month old) podocysts were available. To induce the formation of podocysts, polyps of *Chr. melanaster* were cultured in polystyrene dishes (92x92x18 mm) at either 28 or 18 ^{o}C, fed with excess *Artemia* nauplii (>16.5 at µg C polyp^{-1} d^{-1})

for a month to obtain enough numbers of podocysts, based on results of a preliminary experiment that the podocyst production was higher by well-fed polyps (see below). After the culture for a month, the parent polyps were removed from the dishes, and the remaining podocyts were used in experiments to examine the effect of temperature and dissolved oxygen concentration upon excystment.

Effect of temperature

The podocysts produced by parent polyps at 28 ^{o}C were immediately transferred to lower temperatures (i.e., 19 or 11 ^{o}C), and those at 18 ^{o}C were transferred to higher temperatures (i.e., 22 or 28 ^{o}C) to examine their excystment for 12 weeks. As controls, the podocysts produced at respective temperatures were maintained at the same temperatures throughout the experiment. In addition, the podocysts that were initially at 28 ^{o}C were exposed to lower temperatures in a step-wise fashion (i.e., 25, 22, 19, and 13 ^{o}C, each for 3 weeks), which resembled the seasonal seawater temperature decline. In each treatment, 50-60 podocysts were used by triplicates.

Effect of dissolved oxygen concentration

The podocysts, which had been produced in well-aerated ($\geq$5.0 mg O_2 l^{-1}), 32 salinity seawater at 22 ^{o}C, were subjected to low dissolved oxygen concentration (DO) (target DO: 0.5 mg O_2 l^{-1}) established in DO bottles (volume: ca. 100 ml). The target DO seawater was obtained by purging oxygen by bubbling of nitrogen gas in an 8-l glass bottle, and then the water was gently siphoned into each DO bottle. In order to examine the effect of duration under hypoxic condition, the podocysts were placed in the DO bottles for 1, 3, 7 and 14 days and then returned to the aerated condition to examine excystment for 4 weeks (Table 3). As a control, the podocysts were kept at the initial aerated conditions throughout the experiment. In all treatments,

DO was measured by a DO meter (LDO^{TM} HQ10, Hach Co.) before and after experiment. Thirty podocysts were used in each treatment by triplicates.

Podocyst viability

In order to examine the survivability of unexcysted podocysts of *Chr. melanaster* after DO experiments, the cuticle capsule of which was artificially removed, as had been performed for *A. aurita* podocysts.

Histological studies of podocysts

Histochemistry

Histochemical examination was made using 1-month-old podocysts. The procedures of fixing and cutting of specimens were essentially the same as to *A. aurita*. In order to demonstrate the general morphology, sections (1 μm thickness) were stained with Mayer's Haematoxylin/Eosin technique. In *Chr. melanaster*, however, no histochemical examinations to define the carbohydrate, lipid, DNA and RNA contents were carried out. Further, no examination was made to show the change of internal structure of podocysts with age.

Results

General pattern of podocyst formation

The asexual production by polyps of *Chr. melanaster* was never performed by budding, but only by podocyst production at the base of polyp stalk. In the stock culture conditions during initial 3 months, the podocyst production patterns varied from one polyp to another, and about one third of polyps did not produce podocysts but the rest polyps produced podocysts at least one.

A most productive polyp produced 17 podocysts during 3 months as shown in Fig. 17. This polyp produced the first podocyst, diameter and height of which were ca. 100 and 30 μm, respectively, on 18th day, and produced larger podocysts successively at ca. 7-day intervals (Fig. 18). This polyp produced the largest podocysts (diameter: ca. 900 μm) on 90th day, and stopped producing podocysts. Further observations, although sporadic, revealed that this polyp could survive for nearly 2.5 years under the stock culture condition, but it did not produce any further podocysts.

Over 100 podocysts of *Chr. melanaster* produced by the stock-culture polyps were examined and measured under the microscope. The most of them were irregular circles from the top, with diameter and height varying widely from 100 to 900 μm and from 30 to 150 μm, respectively. The central part of the roof was slightly depressed in most podocysts. Newly produced podocysts were semi-translucent and yellowish in color and the podocysts older than ca. two months became opaque and darker.

Combination effects of temperature and food

Polyps did not produce any podocysts at all in 5 ^{o}C treatments, but all of them stopped feeding and strobilated within a week. At 11 ^{o}C, 90% of polyps produced podocyts before they strobilated, and all of them eventually strobilated by 4 weeks. At this temperature, the average numbers of podocysts produced during 8 weeks of the experiment increased from 0.5 by a non-fed polyp to 4.3 by a polyp with food supply of 16.9 μg C polyp^{-1} d^{-1} (Table 1, Fig. 19). At 18 ^{o}C, a few polyps strobilated during 8 weeks; only one polyp (out of 18) strobilated in 0, 2.4 and 4.8 μg C polyp^{-1} d^{-1} treatments, two polyps (out of 6) in 12.1μg C polyp^{-1} d^{-1} treatment, and three polyps (out of 6) in 16.9 μg C polyp^{-1} d^{-1}. In general, the warmer temperature and the higher food supply, the earlier podocysts formed and the more were formed (Fig. 19). Actually, the

cumulative number of podocysts produced differed significantly with temperature ($F_{5, 150}$=147.230, p<0.001), food supply ($F_{4, 150}$=257.472, p<0.001) and their interaction ($F_{20, 150}$=30.183, p<0.001) (Table 1, Fig. 18). Only few podocysts (i.e. 0.5 podocysts polyp^{-1}) were formed by a starved (i.e. no food supply) polyp at 11^{o}C. The production was highest (i.e. 16.5 podocysts polyp^{-1}) by a polyp with highest food supply (16.9 µg C polyp^{-1} d^{-1}) at 28^{o}C. In a given experimental condition, the podocyst production was relatively constant over 8 weeks, yielding a linear relationship between cumulative numbers of podocysts and duration of experiment (Fig. 20).

Effect of salinity

No polyps could survive at the lowest salinity of 5. Salinity had a significant effect on encystment within the salinity range from 10 to 32 ($F_{5, 30}$=13.636, p=0.001 Table 2, Fig. 21). The podocyst production was lowest (0.5 podocysts polyp^{-1}) by a polyp placed at salinity of 10, and highest (2.3 podocysts polyp^{-1}) by a polyp at salinity of 32. However, there was no significant difference in the average numbers of podocysts within the salinity range from 15 to 32 (p=> 0.05).

Morphology and histology of podocysts

Internal fine structure

A cross section of a-month-old podocyst of *Chr. melanaster* showed that a cell mass is encapsulated by a cuticle cover with thickness of 3-7 µm (Fig. 22A). The dormant cells were stained red strongly with a reaction of the Mayer's Haematoxylin/Eosin technique. There were few small nuclei (ca. 0.3 µm diameter) stained in blue and many granules of various sizes. Cnidoblasts (diameter: 0.7-0.9 µm) also occurred in the dormant cells (Fig. 22B).

General pattern of podocyst excystment

Newly formed podocysts were filled with a whitish-colored cell mass, which gradually shrank to give a notable space between the inner cell mass and the cuticle cover in one-month-old podocysts. The first externally visible event for the excystment was the protrusion of a club-shaped cell mass through an opening hole at the roof of the capsule, and the mass then transformed into a poly with 4 rudimentary tentacles (Fig. 23). The general pattern of the polyp growth after the excystment was almost the same as in *A. aurita*.

Effect of various conditions on podocyst excystment

Effect of temperature

It was notable that podocysts of *Chr. melanaste*r excysted more or less in all temperature treatments (i.e. 28, 25, 22, 19, 18, 13 and 11 ^{o}C). When the podocysts that had been produced at 28 ^{o}C were suddenly transferred to 19 ^{o}C, 34% of them excysted rather rapidly during the initial 4 weeks after the transfer, and thereafter the excystment tended to become slower. After 12 weeks of monitoring, the cumulative excystment increased to 43% (Fig. 24). When the podocysts were transferred suddenly to 11 ^{o}C, a similar pattern of excystment was observed; 33% of podocysts excysted during the initial 8 weeks, but no further excystment occurred later (Fig. 24). In the experiment of stepwise decrease of temperature from 28 to 13 ^{o}C, 14% of podocysts excysted at 25 ^{o}C, and the excystment increased to 19% at 22 ^{o}C, 32% at 19 ^{o}C and finally to 48% at 13 ^{o}C (Fig. 25).

In the experiment where the podocysts, which had been produced at 18 ^{o}C, were suddenly transferred to warmer temperatures of 22 or 28 ^{o}C, fewer excystments occurred compared to the temperature decrease experiment. The cumulative excystment from the polyps kept at 22 and 28

^{o}C was 10 and 8%, respectively (Fig. 26). When the podocysts were kept at either 18 or 28 ^{o}C throughout, only few podocysts excysted, i.e. 11 and 6% at respective temperatures (Fig. 27).

Effect of dissolved oxygen concentration

It was notable that the control podocysts that had been kept in aerated water, 6.7% of them excysted within 6 weeks. The podocyts, which had been kept in deoxygenated condition for 1 and 3 days, did not show any significant difference in the average excystment from the control podocyts (Table 3, Fig. 28). However, the average excystment increased remarkably to 46.7 and 53.3% for the podocysts, which had been kept in hypoxic condition for 7 and 14 days, respectively (Fig. 28). The effect of duration under hypoxia on the podocyst excystment was significant ($F_{4, 10}$=31.571, p<0.000; Fig. 28). It was also worth mentioning that all excystments occurred within one week after returning to well-aerated seawater.

Podocysts viability

When the podocysts were placed at constant temperatures (i.e. 18 or 28 ^{o}C), ca. 100% of podocysts actually excysted (total numbers of podocysts used: 120 and 70, respectively) within 12 months at respective temperatures, indicating that the most of them terminate their dormancy within one year. Therefore, the longevity of podocyts of this species is less than a year.

In order to check the viability of unexcysted podocysts that had been exposed to hypoxia for 1-14 days and returned to aerated condition, their cuticle was removed artificially. The transformation to planura-like cell mass took place in 80-100% of the unexcysted podocysts, indicating that most of them were viable.

Discussion

This study has demonstrated for the first time the effects of various environmental factors on encystment and excystment of the podocysts of *Chr. melanaster*, one of bloom-forming jellyfish species, in addition to general podocyst formation process and some morphological and histological features of the podocysts. First of all, unlike the asexual reproduction pattern of *A. aurita*, podocysts are an exclusive form of asexual reproduction by polyps of *Chr. melanaster*. Although both temperature and food supply were also important environmental factors to determine the podocyst production by *Chr. melanaster* polyps, the food effect was different from the case of *A. aurita*'s podocyst production. The podocyst production was very scarce by polps with no food supply, and increased significantly with the increase of food supply. This result is similar to the podocyst production pattern in *Rho. esculenta* polyps, which produced more podocysts when feeding was more frequent (Guo, 1990).

In previous study with *Chr. quinquecirrha* from Chesapeake Bay, USA, the podocyst formation increased when temperature was cooling toward 2-4 ^{o}C (Cargo & Schultz, 1967). In contrast, *Chr. melanaster* polyps showed higher podocyst formation with the increase of temperature from 11 to 28 ^{o}C, indicating that the asexual reproduction is accelerated at higher temperatures. Within the normal salinity range (15-32) where wild polyps may encounter, the podocyst production by *Chr. melanaster* polyps was not affected by salinity at all.

The general morphology, internal structure and chemical contents of organic reserves of *Chr. melanaster* podocysts were basically the same as those for *A. aurita*, in addition to *Chr. quinquecirrha*, which had been reported by Chapman (1968). Existence of a few nuclei and very weak reaction by RNA in the organic-rich in dormant cells of *Chr. melanaster* podocysts indicated that the basal metabolism of the dormant cells might be low (Black, 1981; Ikeda et al.,

in press). Furthermore, *Chr. melanaster* podocysts could survive under hypoxia (0.5 mg O_2 l^{-1}) for two weeks and maintained the ability of excystment after the hypoxia. Similar tolerance against hypoxia, as well as being buried under organic-rich silty mud, was also been observed for *N. nomurai* podocysts in the laboratory (Kawahara, personal comm.). However, the excystment success of *Chr. melanaster* podocysts kept in the laboratory, even at constant temperatures, was nearly 100% within 12 months, indicating that their maximal dormant period was less than a year.

The excystment of podocysts of *Chr. melanaster* was significantly induced (43-48%) by both sudden and step-wise temperature decrease, although the excystment was significantly low for podocysts which were kept at constant temperatures (11-8% at 18 and 28 ^{o}C) and those that were exposed to temperature increase (8-10%). These results demonstrate that *Chr. melanaster* podocyts are capable of excystment without any specific temperature stimuli, but attain higher excystment when they are exposed to temperature decrease, indicating that their main excystment occurs in autumn. In a similar manner, the excystment of *Cya. capillata* podocysts occurred when water temperature was lowering (Cargo 1974; Brewer & Feingold, 1991). However, the excystment of podocysts of *Rho. esculenta* and *Chr. quinquecirrha* was induced when they were exposed to temperature increasing condition (Cargo & Schultz 1967; Cargo & Rabenold, 1980; Jiang et al., 1993). The exposure to hypoxic condition (i.e. 0.5 mg O_2 l^{-1}) for at least one week has a striking effect to induce the excystment of *Chr. melanaster* podocyts. This result suggests that *Chr. melanaster* podocysts can survive under hypoxic condition, which is often created in eutrophic coastal waters in summer and then excyst when the summer hypoxia ends in autumn.

Based on previous reports on the seasonal occurrence of medusae and the results of asexual reproduction obtained in this study, the seasonal life cycle of *Chr. melanaster* in east

Asian coastal waters is schematically presented in Fig. 29. The sexual reproduction by *Chr. melanaster* medusae may occur mainly in spring (e.g. March-June), since fully-grown medusae of average diameter of ca. 25 cm appear in spring and become senescent to disappear from the plankton in summer in the Inland Sea of Japan (Uye & Ueta, 2004; Ueda, 2001; Makabe et al., unpublished data). A similar seasonal occurrence was also reported for *Chr. melanaster* in Tokyo Bay (Nomura & Ishimaru, 1998; Kinoshita et al., 2006). Hence, it is in spring that the sexually-produced planula larvae are produced and the planulae attach to some hard substrates to metamorphose to polyps, entering the benthic phase of the life cycle, which may be perennial. In summer, due to increased temperature and perhaps higher food supply, the polyps accelerate the production of podocysts. In autumn, the podocyst production gradually decreases as the temperature decreases, whilst many podocysts are induced to excyst due to temperature-lowering stimulus and concurrent recovery from summer hypoxia. In early winter, these excysted polyps start transformation to strobilae due to additional cooling of temperature. Ephyrae are released to the water, and they grow gradually during winter and attain sexual maturity in spring. Few podocysts remain longer than a year.

The value of the podocysts in the life cycle of *Chr. melanaster* lies in the increase of polyp numbers as well as dormancy by which the population endure unfavorable conditions (i.e. hypoxia, low food supply, predation by predators). Hence, the performance of podocysts may play very important roles in population dynamics of this scyphozoan jellyfish species. However, compared to the asexual reproduction of *A. aurita* polyps by budding, the reproductive potential of *Chr. melanaster* by podocyst production, an exclusive means of asexual reproduction, is apparently lower. In addition, in terms of dormant ability, or durability, *Chr. melanaster*

podocysts may apparently be inferior (less than a year) to *A. aurita* podocysts, which can persist for 3.2 years.

Chapter 4. Asexual reproduction of *Cyanea nozakii* with special reference to the role of podocysts

Introduction

It is known well that the life cycle of most scyphozoan species consists of two stages: a conspicuous pelagic stage and a less conspicuous benthic one (e.g. Agassiz, 1862; Mayer, 1910; Thiel, 1959, 1962; Russell, 1970; Arai, 1997). Hence, in order to comprehend the seasonal occurrence of medusa population (e.g. timing and duration of the occurrence, and geographical distribution and abundance of the population), it is necessary to understand the process of asexual reproduction undertaken by benthic polyp stage (i.e. budding, fission and podocyst production), as have already been described in *A. aurita* and *Chr. melanaster* in the previous chapters. However, the scyphozoan benthic process is a bit more complicated. In the genus *Cyanea*, there is an encystment stage called planulocyst by immediately attached planulae (Brewer, 1976). Although the planulocysts may protect themselves from adverse conditions such as extreme physico-chemical environmental factors, potential competitors for settlement and predators (Brewer & Feingold, 1991; Holst et al., 2007), their mortality is high due to infestation of sessile organisms such as algae, barnacles, bryozoans, hydrozoans, and ascidians (Gröndahl, 1988; Colin & Kremer, 2002). These facts may indicate that the planulocysts are less robust compared to podocysts, which are also produced by polyps of the genus *Cyanea.* There are two previous studies that have investigated the effect of temperature on the podocyst formation and excystment in the genus *Cyanea*; one is *Cya. capillata* from Chesapeake Bay, USA (Cargo, 1974), and another is *Cyanea* sp. from Connecticut, USA (Brewer & Feingold, 1991).

Jellyfish of the genus *Cyanea*, which consist of 14 species (Kramp, 1961; Mianzan & Cornelieus, 1999), are distributed widely in world coastal seas. *Cya. capillata* and *Cya. lamarckii* Péron & Lesueur are common in temperate and boreal zones, (Cargo, 1987; Gröndahl, 1988; Brewer & Feingold, 1991; Holst & Jarms, 2010) and they are predators of not only zooplankton and fish but also other jellyfish species (Bamstedt et al., 1994; Purcell & Arai, 2001). It is also known that accidental contacts with *Cyanea* species often make humans cutaneous irritations and pain, and occasionally cardiovascular system failure (Burnett, 2001). In Loch Fyne, west coast of Scotland, salmon farming has destructed by repeated *Cya. capillata* attacks, which killed thousands of salmon when their invasion was massive (Purcell et al., 2007).

A temperate species, *Cya. nozakii,* is common in coastal waters along the east Asian countries. In Chinese waters, this species used to appear only sporadically in the past, but it has become widely distributed and more abundant within a short time around 2004, in associated consequences of habitat loss, eutrophication and overfishing (Dong et al., 2010). When blooming, the medusae damage fisheries by breaking fishing nets (Dong et al., 2008). In the Inland Sea of Japan, this species occasionally occur so abundantly as to hamper fisheries because of large body biomass, strong stickiness and venomous sting (Uye & Ueta, 2004).

The life cycle of *Cya. nozakii* has been described by Dong et al. (2006) for specimens collected from Chinese coastal water, but the benthic stage has not been investigated in detail. Hence, I have conducted laboratory experiments to study the asexual reproduction of *Cya. nozakii* polyps, and, in particular, I have examined the effects of various environmental factors (i.e. temperature, salinity, food supply, dissolved oxygen concentration) on encystment and excystment, following similar procedures used in *A. aurita* and *Chr. melanaster*.

Materials and methods

Effect of various conditions on podocyst formation

Preparation of polyps

Mature medusae of *Cya. nozakii* were caught in Aki-nada, central Inland Sea of Japan, in September 2010. The gonadal fragments (ca. 500 g) from the medusae were cut off and transported to a laboratory at Hiroshima University. Small pieces of ovary and testis (ca. 10 g) from 3 to 5 specimens were placed together in plastic vessels containing filtered seawater of 32 salinity at 18 ^{o}C overnight in darkness. Then, fertilized eggs and swimming planulae were isolated with a pipette under a dissecting microscope and transferred to plastic vessels (diameter: 130 mm, depth: 55 mm) containing filtered seawater to settle. The vessels were kept in the dark at 22 ^{o}C, and newly transformed polyps were fed with a batch of newly hatched nauplii of *Artemia* sp. (Utah, USA) at 2 to 3 day intervals to establish the stock cultured of polyps.

In November 2010, the polyps of ca. 2 months old with average stalk diameter of 150 μm, which had not produced any podocysts, were removed gently from the wall of stock-culture containers using a thin metal blade and placed individually in wells of 6-well polystyrene culture plates, each containing 10 ml of filtered seawater of salinity 32. The plates were kept at 22 ^{o}C for one week in darkness to ensure attachment to the well bottom until the start of podocyst formation experiments. Following the experimental procedures used in *A. aurita* and *Chr. melanaster,* the combined effects of temperature and food supply and the effect of salinity on podocyst formation by *Cya. nozakii* polyps were examined.

Combination effects of temperature and food supply

As shown in Table 1, the experiment consisted of total of 30 different combinations of temperatures (6 levels) and food supply (5 levels) using well-aerated (≥5.0 mg O_2 l^{-1}), 32 salinity seawater as had been conducted in *A. aurita* and *Chr. melanaster.*

Effect of salinity

As shown in Table 2, the method was essentially the same as had been used in *A. aurita* and *Chr. melanaster*.

Effects of various conditions on podocyst excystment

Preparation of podocysts

For this experiment, only young (≤1 month old) podocysts, which had been produced in polystyrene dishes at either 28, 22 or 18 ^{o}C, fed with excess *Artemia* nauplii (>16.5 at µg C $polyp^{-1}$ d^{-1}), were used.

Effect of temperature

The podocysts produced by parent polyps at 28 ^{o}C were immediately transferred to lower temperatures (i.e., 19 or 11 ^{o}C), and those at 18 ^{o}C were transferred to higher temperatures (i.e., 22 or 28 ^{o}C) to examine their excystment for 12 weeks. The control podocysts were also prepared. In addition, the podocysts that were initially at 28 ^{o}C were exposed to lower temperatures in a step-wise fashion (i.e., 25, 22, 19, and 13 ^{o}C, each for 3 weeks). In each treatment, 30-40 podocysts were used by triplicates.

Effect of dissolved oxygen concentration

The podocysts, which had been produced in well-aerated (≥5.0 mg O_2 l^{-1}), 32 salinity seawater at 22 ^{o}C, were subjected to low dissolved oxygen concentration (DO) (target DO: 0.5

mg O_2 l^{-1}) established in DO bottles (volume: ca. 100 ml). In order to examine the effect of duration under low DO, the podocysts were placed in the DO bottles for 1, 3, 7 and 14 days and then returned to the aerated condition to examine excystment for 4 weeks (Table 3). The control was also prepared. Thirty podocysts were used in each treatment by triplicates.

Podocyst viability

In order to examine the viability of unexcysted podocysts after the DO experiment, the cuticle was removed. In order to examine the maximum longevity of podocysts, the cuticle of the podocysts that had been kept at 22 oC for various durations were removed.

Histological studies of podocysts

Histochemistry

Histochemical examination was made using 1-month-old podocysts. The procedures were essentially the same as used in *A. aurita* and *Chr. melanaster*.

Results

General pattern of podocyst formation

The polyps of *Cya. nozakii* never performed asexual production by budding, but only by podocyst production (Fig. 30). In the stock culture conditions for two months, there were marked differences in podocysts production from one polyp to another, and more than 70% of polyps did not produce podocysts. One of the most productive polyps produced 6 podocysts within three months under stock culture condition as showed in Fig. 30. The pattern of podocyst formation was similar to *Chr. melanaster*, the first podocyst was produced on 35th day, with diameter and height of ca. 70 and 30 μm, respectively, and podocysts were successively produced at ca. 7-day

intervals up to three months (Fig. 31). The diameter of the podocysts became larger up to ca. 200 μm (Fig. 31). Although this polyp is still surviving at present (for ca. one year), it did not produce any further podocysts under the stock culture condition. The podocysts of *Cya. nozakii* were yellowish in color, round in top view (diameter range: 70-200 μm) and trapezoid in side view (height rage: 30-50 μm), and the central part of the roof is slightly concaved (Fig. 35A).

Combination effect of temperature and food supply

At 5 and 11 ^{o}C, polyps did survive, but they did not produce any podocysts at all. None of them strobilated at all temperatures during 8 weeks of experiment. The podocyst production was made at 18 ^{o}C, and the average numbers of podocysts produced during the experiment were lowest (0.3) by a starved polyp and increased to 4.3 by a polyp with food supply of 16.9 μg C polyp^{-1} d^{-1}. In general, the warmer temperature, the earlier podocysts formed and the more were formed (Figs. 32). Actually, the numbers of podocysts produced differed significantly with temperatures ($F_{5,\ 150}$=366.674, p<0.001), food supply ($F_{4,\ 150}$=185.529, p<0.001) and their interaction: ($F_{20,\ 150}$=22.464, p<0.001; Table 1). However, the average podocyst production at higher temperatures of 22, 26 and 28 ^{o}C did not differ significantly. In a given experimental condition, the podocysts production was relatively constant over 8 weeks, yielding a linear relationship between cumulative numbers of podocysts and duration (Fig. 33).

Effect of salinity

At the lowest salinity of 5, all polyps died within a week. At salinity10, the tentacles of polyps shrank, and the polyps stopped feeding to die off 2 weeks after the experiment without producing any podocysts. Although the maximum production (average: 2.0 podocysts polyp^{-1}) was attained at salinity 20, there was no significant difference in the average podocyst production among salinities ranging from of 15 to 32 ($F_{4,\ 25}$=0.637, p=0.641; Table 2, Fig. 34).

Morphology and histology of podocysts

Internal fine structure

One-month-old podocysts of *Cya. nozakii* contained a mass of various shaped and sized cells (Fig. 35A). A small nucleus (ca. 0.1 μm in diameter) occurred in each cell, and cnidoblasts (ca. 0.3 μm) occurred in the cell mass (Fig. 35B). The cuticle layer was thinner at the roof (2-3 μm) than that in the lateral (4-6 μm) and bottom (3-6 μm) parts.

General pattern of podocyst excystment

Newly-produced podocysts of *Cya. nozakii* were filled with whitish colored cell mass. The cell mass gradually shrank to make a visible space between inner cell mass and cuticle cover ca. a month later. The excystment occurred by protruding a club-shaped cell mass from the opening of the upper part of podocyst. About 1-3 days later, 4 rudimentary tentacles were formed (Fig. 36). The general pattern of polyp development after the excystment was the same as in *A. aurita* and *Chr. melanaster.*

Effects of various conditions on podocyst excystment

Effect of temperature

Excystment never occurred when the podocysts were exposed to temperature increase from 18 ^{o}C to 22 or 28 ^{o}C. When the podocysts, which had been produced at 28 ^{o}C, were suddenly transferred to 19 ^{o}C, 25% of them excysted within 2 weeks, 56% of them excysted within 10 weeks, and no further excystment occurred thereafter (Fig. 37). When the podocysts were transferred to 11 ^{o}C, 32% of them excysted within 2 weeks and 65% within 8 weeks, and no further excystment thereafter (Fig. 37).

In the experiment of stepwise decrease of temperature from 28 to 13 ^{o}C, 3% of podocysts excysted at 25^{o}C, and the excystment gradually increased to 53% at 13 ^{o}C (Fig. 38). No excystment occurred in the control at constant temperatures at 28 or 18 ^{o}C.

Effect of dissolved oxygen concentration

No excystment occurred for control podocysts which had been kept in aerated conditions throughout. The podocysts, that had been exposed to hypoxic condition and returned to normal DO condition, excysted at higher success (range: 53.3-63.3%) within 10 days. Hypoxic duration did not affect significantly on the excystment of podocyst ($F_{3, 8}$=0.425, p>0.725; Table 3, Fig. 39).

Podocyst viability

When the cuticle was removed from unexcysted podocysts after the DO experiments, 80% of them successfully transformed into polyps indicating that most of them were viable. Cuticle removal has confirmed that one-year-old podocysts kept at 22 C are still viable.

Discussion

Characteristic features in the asexual reproduction of *Cya. nozakii* lie in the exclusive production of podocysts, delayed strobilation by relatively old polyps kept at warm temperatures, and the formation of monodisc strobilae (Dong et al., 2006). The podocysts production was influenced not only by temperature but also food supply, as had been found in *A. aurita* and *Chr. melanaster*. In *Cya. capillata* from Chesapeake Bay and Niantic River Estuary, podocysts were produced during a temperature-increasing period (Cargo, 1987; Brewer & Feingold, 1991). Similarly, the podocyst production in *Cya. nozakii* increased with the increase of temperature,

although the temperature effect was not so significant as the podocysts production was no longer accelerated when the temperature increased from 22 to 28 ^{o}C, suggesting that some temperature saturation for *Cya. nozakii* polyps. The podocyst production increased with the increase of food supply, but the production was no further accelerated at food regimes higher than 4.8 μg C polyp^{-1} d^{-1}, suggesting that the existence of food satiation for polyps to produce podocysts. Low salinity levels of 5 and 10 were apparently lethal or adverse to *Cya. nozakii* polyps. However, within the salinity range from 15 to 32, the podocyst production was not affected by change of salinity.

The podocysts of *Cya. nozakii* were highly induced to excyst (52-65%), when they were exposed to lower temperatures in both sudden and stepwise manners. These results were similar to *Cya. capillata*, the podocysts of which also excysted when they were exposed to lowering temperature (Cargo, 1974; Brewer & Feingold, 1991). On the contrary, no excystment occurred for podocysts of *Cya. nozakii* kept at constant temperatures or those exposed to temperature increase. Furthermore, the effect of hypoxia was also striking to induce excystment of *Cya. nozakii* podocysts; the exposure to hypoxia only for one day was effective.

The general morphology and internal structure of *Cya. nozakii* were more or less similar to those of *A. aurita* and *Chr. melanaster*. A confirmation was made that *Cya. nozakii* podocysts can survive at least one year. In *Chr. quinquecirrha,* the podocysts were viable for as long as 25 months (Black et al., 1976). Hérouard (1911) reported that the maximum longevity of *Chr. hysocella* podocysts was more than 3 years.

The seasonal life cycle of *Cya. nozakii* in east Asian waters is schematically presented in Fig. 40, based on previous reports on the seasonal occurrence of medusae and the results of this study. Dong et al. (2006, 2008) reported that the number of *Cya. nozakii* increased significantly

after the end of July in Chinese coastal seas. A similar seasonal occurrence was also observed in the Inland Sea of Japan, where medusae became larger in size and encountered more frequently in July and became senescent in October. Hence, the sexual reproduction by *Cya. nozakii* medusae may occur mainly in summer (e.g. July-September). Sexually produced planulae may attach to benthic substrates to transform polyps, but some of them may form planulocysts. Concurrently, the podocysts are formed and accumulated. As water temperature decreases in autumn, the podocyst production reduces and then stops. At the same time, the podocysts may start excystment to new polyps to enhance the polyp population. Excystment is also induced from the podocysts, which might had been exposed to summer hypoxia, because the seasonal vertical mixing alleviates the benthic hypoxia. It is worth mentioning that those excysted polyps may not transform to strobilate but may remain in polyp stage throughout winter season. This was confirmed in podocyst production experiments, where polyps did not transform to strobilae at temperatures of 5 and 11 ^{o}C, in addition to observation of monodisc strobilation by 6-month-old polyps (ca. 10%) kept at 22 ^{o}C. A similar strobilation timing was also reported by Dong et al (2006) for *Cya. nozakii* from Chinese waters, the polyps of which strobilated at temperatures of 22 and 26 ^{o}C. Hence, the strobilation is expected to occur when the temperature increases to around 20 ^{o}C, (i.e. in early summer). Soon, ephyrae are released to the waters to grow gradually to attain sexual maturity in summer. This seasonal life cycle pattern is different from that of *Cya. lamarckii* in Chesapeake Bay, USA whose strobilation takes place in autumn when temperature is decreasing (Delap, 1905; Cargo & Schultz, 1967; Cargo, 1974; Gröndahl & Hernroth, 1987), and from the seasonal life cycle of *Cya. capillata* in the Baltic Sea (Holst & Jarms, 2010).

In conclusion, the roles of podocysts may lie in two ways in the life cycle of *Cya. nozakii* to maintenance of their population: one is the contribution to increasing the polyp population

abundance and the other is the dormancy by which the population can endure for unfavorable environmental conditions. Nevertheless, the role of podocysts as a means for the medusa population increase may be of least importance among *A. aurita, Chr. melanaster* and *Cya. nozakii*, since the podocyst production rate is lowest and the disc number produced per strobila is only one.

Chapter 5. General discussion

There are plenty of publications reporting on jellyfish population increase and population outbreaks, or blooms, in the marine ecosystems worldwide in recent decades. In the east Asian seas around Japan, China and Korea, where the anthropogenic impact on the coastal marine ecosystem is one of the strongest in the world, the occurrence of the scyphozoan jellyfish blooms has become more extensive and frequent than before. The blooming species include *A. aurita, Chr. melanaster* and *Cya. nozakii,* which tend to occur in regional coastal waters, in addition to *N. nomurai*, which is distributed extensively over the entire east Asian seas. Since it has been widely recognized that the asexual reproduction during benthic polyp stage is a key to determine the medusa population abundance in the following season, the numbers of studies dealing with polyps have increased in recent years. It is more than a century ago that Hyde (1894) found the existence of podocysts as one of the asexual reproduction modes of jellyfish. Since then, however, only a few studies have been conducted to reveal the morphological and physio-ecological properties of the podocysts. In the light of recent increase of scyphozoan jellyfish blooms, it is required to investigate this characteristic benthic life stage in order to understand the ecological roles in the population dynamics of bloom-forming jellyfish (Arai, 2009). To fulfill this scientific necessity, here I used three bloom-forming scyphozoan species, viz. *A. aurita, Chr. melanaster* and *Cya. nozakii,* and investigated the effects of various environmental factors on encystment and excystment of their podocysts.

Some knowledge on the podocysts of the giant jellyfish *N. nomurai* has become available from recent studies by Kawahara et al. (2006) and Ikeda et al. (in press). In this species, the podocyst production is a sole means of asexual reproduction by polyps, and new polyps are excysted from the podocysts (Kawahara et al., 2006). The production of podocysts is generally

low; the maximum number of podocysts produced was 16 by a polyp kept at 23°C for 6 months (Kawahara, personal comm.). The production is accelerated with the increase of temperature, but it is not necessarily accelerated under excess food condition (Kawahara et al., 2006; Sekiya, personal comm.). The excystment occurs when podocysts are placed in rather abnormal environmental conditions such as high temperature, low salinity and hypoxia (Kawahara et al., 2006; Ikeda et al., in press). Although the outer morphology and inner fine structure of *N. nomurai* podocysts are essentially the same as in the other species, they are capable of dormancy for at least 5 years (Ikeda et al, in press). The polyps transform to poly-disc strobilae, from which ephyrae are released into the water in late spring and early summer, when water temperature is around 15 °C (Kawahara et al., 2006).

General pattern of podocyst formation

First of all, there are several modes of asexual reproduction by polyps of *A. aurita,* viz. budding, longitudinal fission and podocyst formation (Arai, 1997, 2009; Vagelli, 2007; Han & Uye, 2010). In both *Chr. melanaster* and *Cya. nozakii*, however, the podocyst formation is an exclusive form of asexual reproduction undertaken by polyps. The podocysts consist of an outer cuticle cover, which is made of protein-chitin complex (Chapman, 1968, 1970; Blanquet, 1972; Black et al., 1976; Black, 1981), and an inner cell mass. Their outer morphology looks similar each other, i.e. irregular circles in top view and trapezoid in lateral view, but there is a slight difference at the roof, being dome-shaped in *A. aurita* and slightly depressed in the others. The coloration is more or less similar each other; young podocysts are semi-translucent and whitish or yellowish and podocysts older than ca. two months become opaque and darker.

According to histological studies, only a few nuclei and very weak reaction by RNA are found in the organic-rich in dormant cells of podocysts in all three species, indicating that basal metabolism of the dormant cells is low (Black et al., 1981; Ikeda et al., in press). The detailed study using *A. aurita* podocysts revealed that the dormant cells are filled with granules containing carbohydrates, proteins and lipids, but the initial reserves may have consumed during dormant period; about a half of them has gone one year later. Although a similar study to examine the temporal change in the cellular nutritional reserves was not performed in *Chr. melanaster* and *Cya. nozakii*, the consumption of the reserve may be quickest in *Chr. melanaster* podocysts, because of their shortest dormant period (less than a year). On the other hand, Ikeda et al., (in press) found that there is no significant decrease in both cell mass volume and nutritional reserves during 5 years of dormancy in the podocysts of *N. nomurai*.

Since the podocyst production is an exclusive form of asexual reproduction in *Chr. melanaster* and *Cya. nozakii*, in addition to *N. nomurai*, the podocysts certainly play a significant role in boosting the polyp population abundance. The maximum podocyst production by a well-fed *Cya. nozakii* polyp was 0.75 podocysts polyp^{-1} week^{-1} (Table 1), which is lower than the maximum production by a well-fed *Chr. melanaster* polyp (i.e. 2.1 podocysts polyp^{-1} week^{-1}, Table 1). Additionally, in *N. nomurai,* the average podocyst production rate is as low as ca. 0.4 podocysts polyp^{-1} week^{-1} (Kawahara, personal comm.). Meanwhile, in *A. aurita*, the podocyst production is not a means for the increase of polyp population abundance but apparently for refuge of the population from some unfavorable conditions. Hence, the budding is a regular means to increase the polyp population in this species. The maximum budding rate of *A. aurita* (i.e. 8.1 buds polyp^{-1} week^{-1}, Han & Uye, 2010) is much higher than the podocyst production rates of the other species. It was also observed that the podocyst production by *Chr. melanaster*

and *Cya. nozakii* is primarily confined to initial 3-6 months, but the budding by *A. aurita* is more or less continuous at least ca. one year (Han & Uye, unpublished data). In addition, *Cya. nozakii* polyps transform to only mono-disc strobilae, but the other species transform to poly-disc strobilae. The species-specific characteristics in the asexual reproduction are listed in Table 5, and based on these data; I can conclude that the potential of the population increase by means of asexual reproduction during the benthic polyp (including strobilation) stage is highest in *A. aurita*, followed by *Chr. melanaster* and *Cya. nozakii*, and lowest in *N. nomurai*.

Effects of various conditions on podocyst formation

The podocyst production rates in *A. aurita*, *Chr. melanaster* and *Cya. nozakii*, were uniformly affected in positive manner with the increase of temperature, demonstrating that the podocyst production is actively performed primarily in warm seasons. This also suggests that the recent global warming may have accelerated the podocyst production rate than used to be.

There was a clear difference in the effect of food supply on podocyst production among species; the production was affected positively with the increase of food supply in both *Chr. melanaster* and *Cya. nozakii,* but negatively in *A. aurita*. This fact is reasonable by taking the podocyst production as a solo reproductive means in the latter two species into consideration. In *A. aurita,* however, the podocyst production is only confined by starved or scarcely-feeding polyps, indicating that the podocysts are produced for refuge of the population. In these three species, salinity did not affect the podocyst production within the normal range (15-32) where polyps may usually encounter in the field.

Effects of various conditions on podocyst excystment

It is interesting to note that the excystment was induced when the podocysts of these three species were exposed to cooling temperatures, indicating that the excysted polyps are recruited to the polyp population in autumn. The podocysts of *A. auriat* and *Cya. nozakii* excysted very rarely when they were kept at constant or warming temperatures, but *Chr. melanaster* podocysts excysted more commonly under the constant temperature conditions. In addition, nearly 100% of *Chr. melanaster* podocysts completed excystment less than a year, indicating that their dormancy as well as durability is lesser than the other species.

Being exposed to hypoxic condition and subsequent placement in aerated conditions induced excystment of podocysts in similar manner in these three species, indicating that the excystment is also enhanced if the podocysts are exposed to summer hypoxia and subsequent recovery by autumnal water mixing.

Role of podocysts in the seasonal medusa occurrence and blooming

The seasonal life cycle of *A. aurita, Chr. melanaster* and *Cya. nozakii* in temperate east Asian coastal waters, consisting of both pelagic medusa stage and benthic polyp stage, is schematically represented in Figs. 16, 28 and 40, respectively, with special emphasis on the role of podocyts. The present study demonstrated that the podocysts play important roles in the seasonal population dynamics of these three semeostome jellyfish species, as had already been surmised in a rhizostome species *N. nomurai* (Kawahara et al., 2006; Ikeda et al., in press). The major ecological role of the podocysts may lie in two aspects; one is, of course, to increase the polyp population abundance and another is for a refuge to protect the population from extinction by unfavorable environmental conditions. As podocysts are exclusive forms of asexual

reproduction by *Chr. melanaster*, *Cya. nozakii* and *N. nomurai,* the above-mentioned two ecological significances of the podocysts are equally important in these species. However, in *A. aurita*, podocysts are produced only by starved and scarcely-fed polyps, the ecological role of podocysts may be much more important as a strategy for population refuge to overcome unfavorable environmental conditions. In this species, budding is a common mode of asexual reproduction and the rate of budding is much higher than that of the podocyst production. The difference in types of strobilation affects the increase of the medusa population abundance; it is poly-disc (range: ca. 5-15 discs per strobila) in *A. aurita* and *Chr. melanaster*, and mono-disc in *Cya. nozakii*. Hence, based on the above-mentioned modes and rates of asexual reproduction by polyps, the potential to increase the medusa population abundance, or to cause jellyfish bloom, is highest in *A. aurita*, followed, in order, by *Chr. melanaster*, *Cya. nozakii* and *N. nomurai*. This agrees well with the facts that the population outbreak of *A. aurita* has become increasingly prominent recently in many eutrophic coastal seas worldwide. As far as eutrophic coastal waters of temperate east Asian area are concerned, the occurrence of *Chr. melanaster* and *Cya. nozakii* medusae is much less abundance compared to *A. aurita*. However, conspicuous blooms of *N. nomurai* medusae over extensive east Asian seas cannot be explained solely by the reproductive potential of these species polyps, other factors may also be involved. Since the basic biological features specific to each jellyfish species are influenced by environmental conditions, primarily temperature, food supply and hypoxia, concomitant studies both in controlled laboratory experiments and in situ surveys on the environmental variables as well as polyp and medusa population dynamics are always necessary in order to understand the mechanisms to cause jellyfish blooms.

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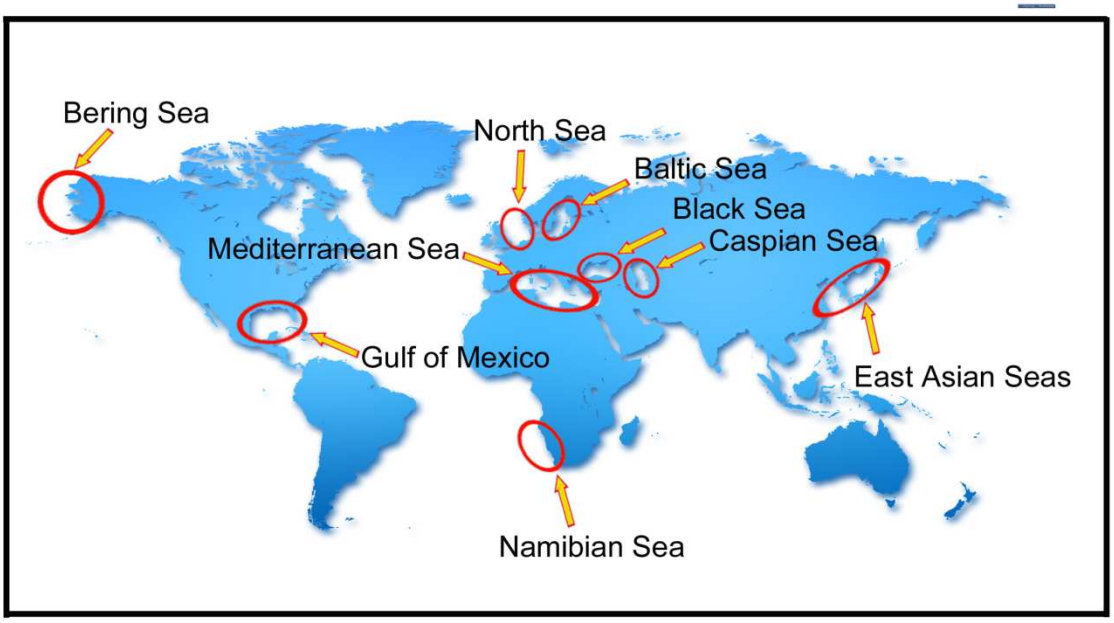

Fig. 1. A global map showing problematic jellyfish blooms.

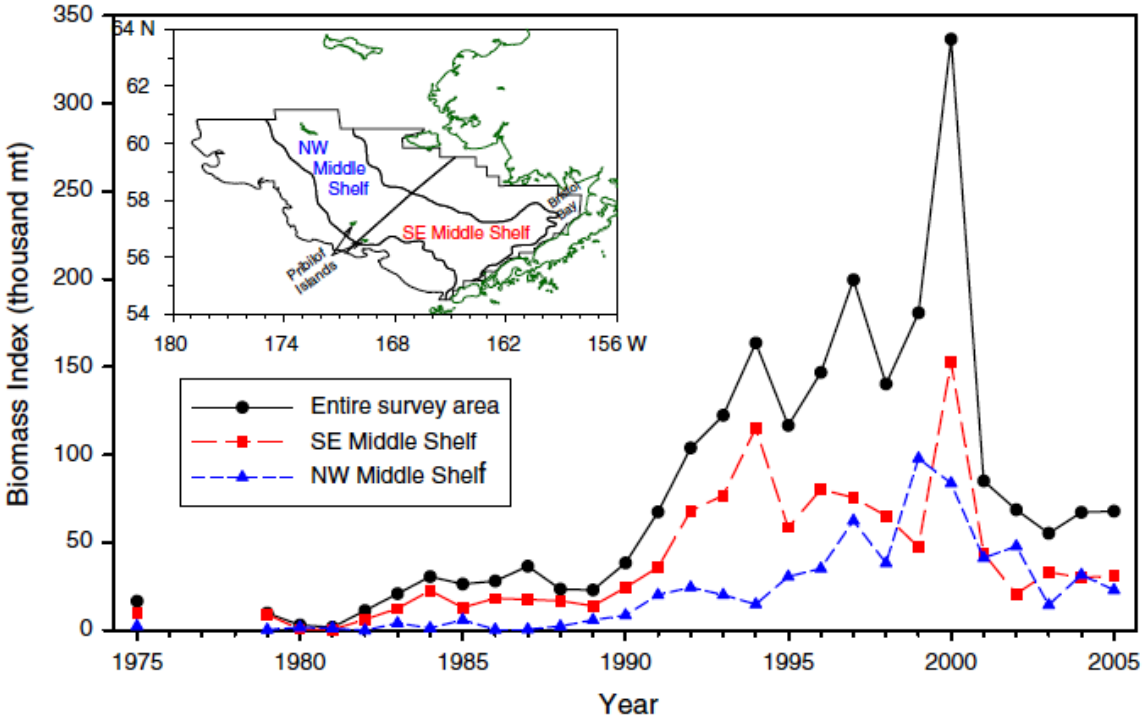

Fig. 2. Trend in jellyfish biomass from standardized trawl surveys in the Bering Sea since 1975. The inset shows the sampling areas on the Bering Sea shelf (Brodeur et al., 2008).

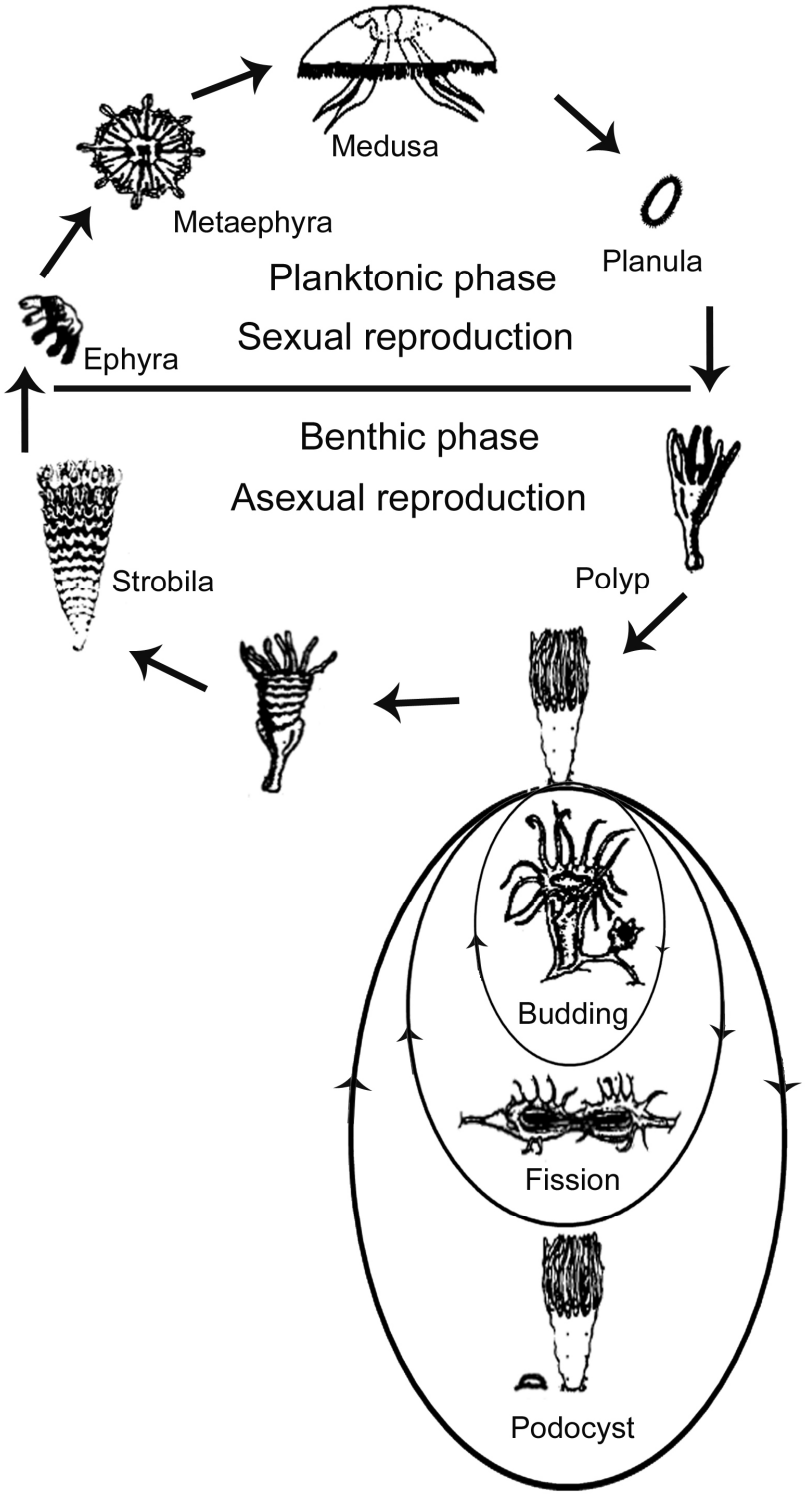

Fig. 3. Schematic diagram to show the life cycle of *Aurelia aurita*.

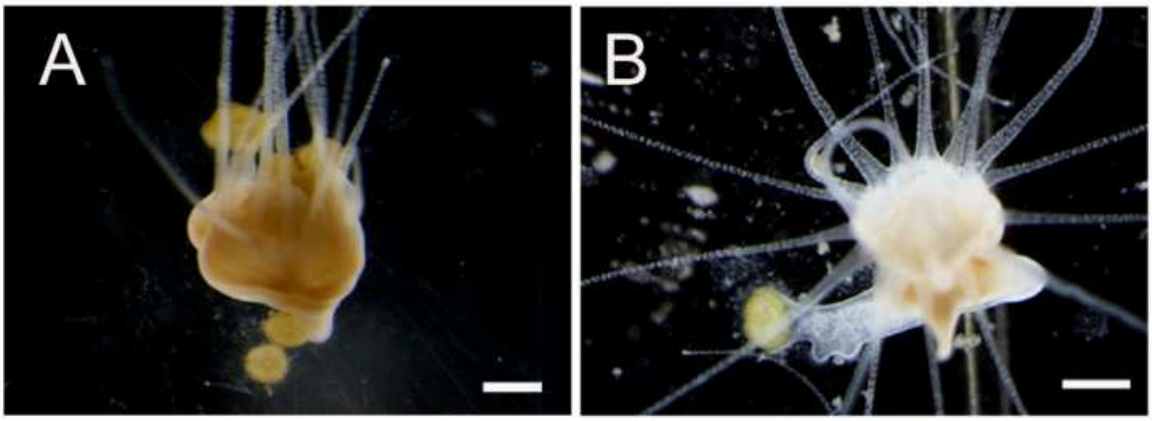

Fig. 4. Formation of *Aurelia aurita* podocysts at the base of polyp stalk (A) and at the end of extended stolon (B). Scale bars = 500 µm.

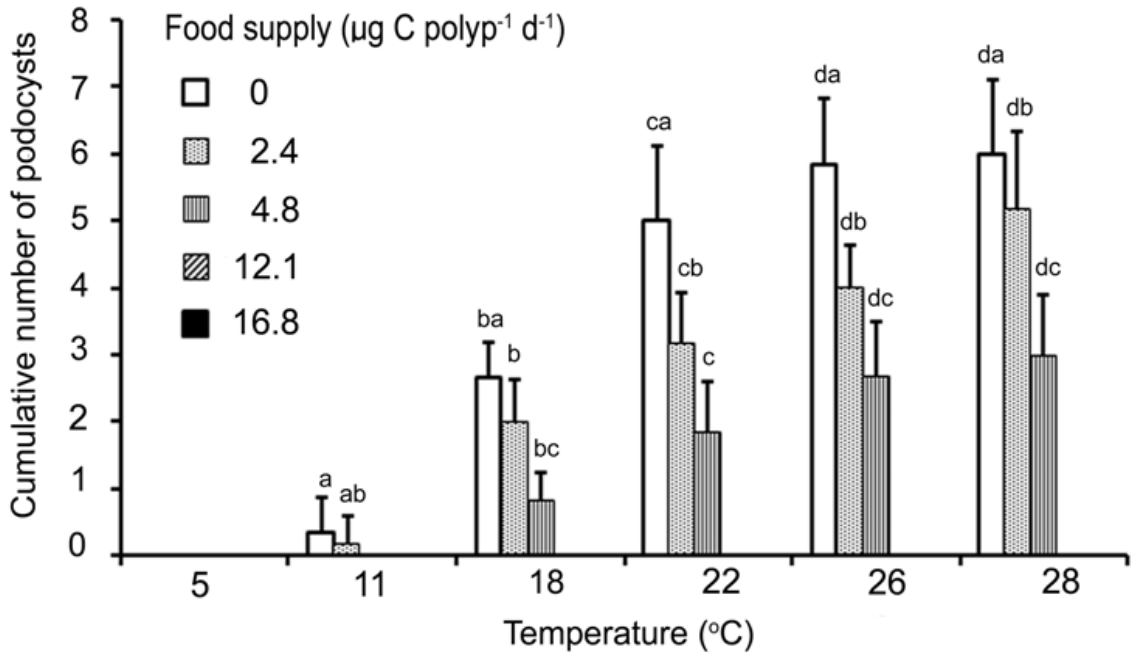

Fig. 5. Cumulative numbers of *Aurelia aurita* podocysts produced during 8 weeks under each combination of 6 temperatures and 5 food supplies. Salinity and dissolved oxygen concentration were 32 and ≥5.0 mg O_2 l^{-1}, respectively. Vertical bars denote standard deviations. Means with different letters are significantly different.

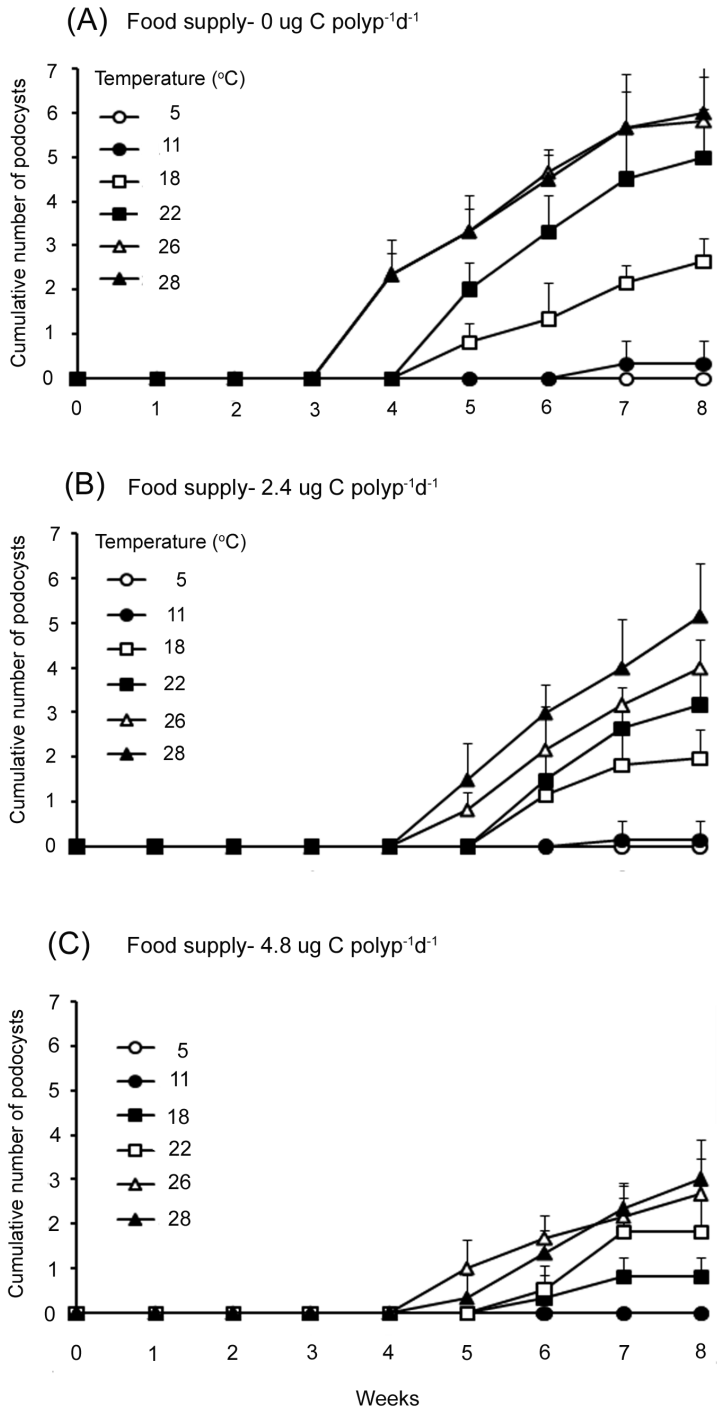

Fig. 6. Cumulative number of *Aurelia aurita* podocysts produced at 6 different temperatures at 0 (A), 2.4 (B) and 4.8 µg C $polyp^{-1}d^{-1}$ (C) during 8 weeks of experiment. Vertical lines denote standard deviations.

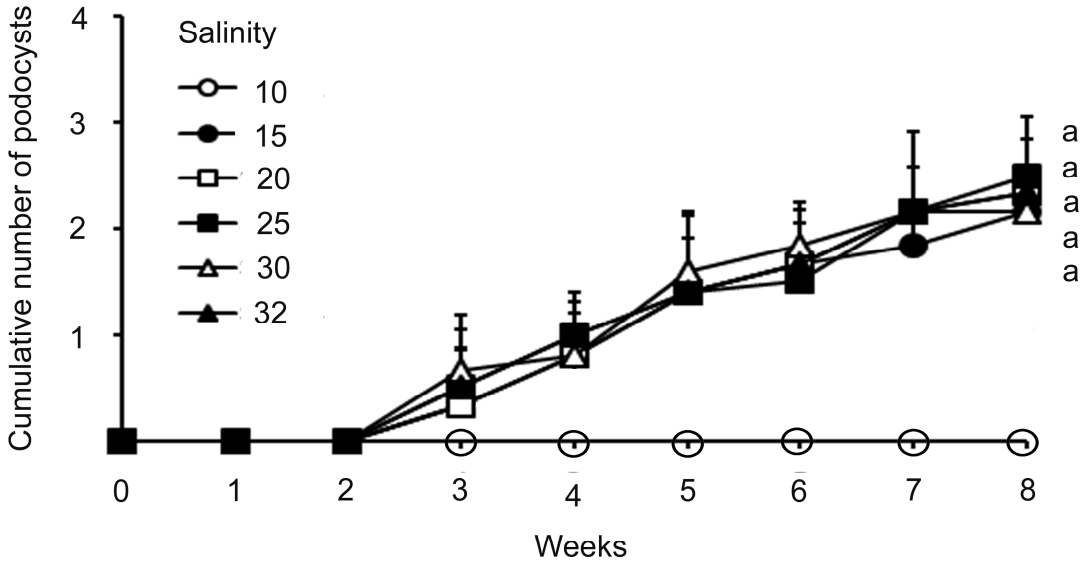

Fig. 7. Cumulative numbers of *Aurelia aurita* podocysts produced during 8 weeks at different salinities. Temperature, food supply and dissolved oxygen concentration were 22 ^{o}C, 2.4 µg C polyp^{-1}d^{-1} and ≥5.0 mg O_2 l^{-1}, respectively. Vertical bars denote standard deviations. Means with different letters are significantly different.

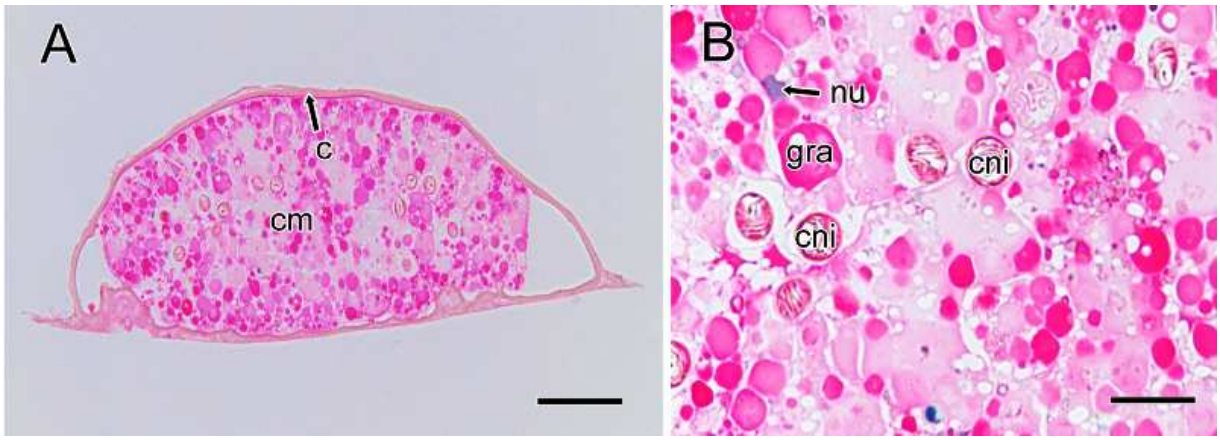

Fig. 8. Photomicrograph of 1-month-old *Aurelia aurita* podocysts stained with Haematoxylin-eosin method. (A) Cross section. (B) Magnified view of nucleus and cnidoblasts in cell mass. c, cuticle; cm, cell mass; cni, cnidoblast; nu, nucleus. Scale bars=50 μm (A) and 10 μm (B).

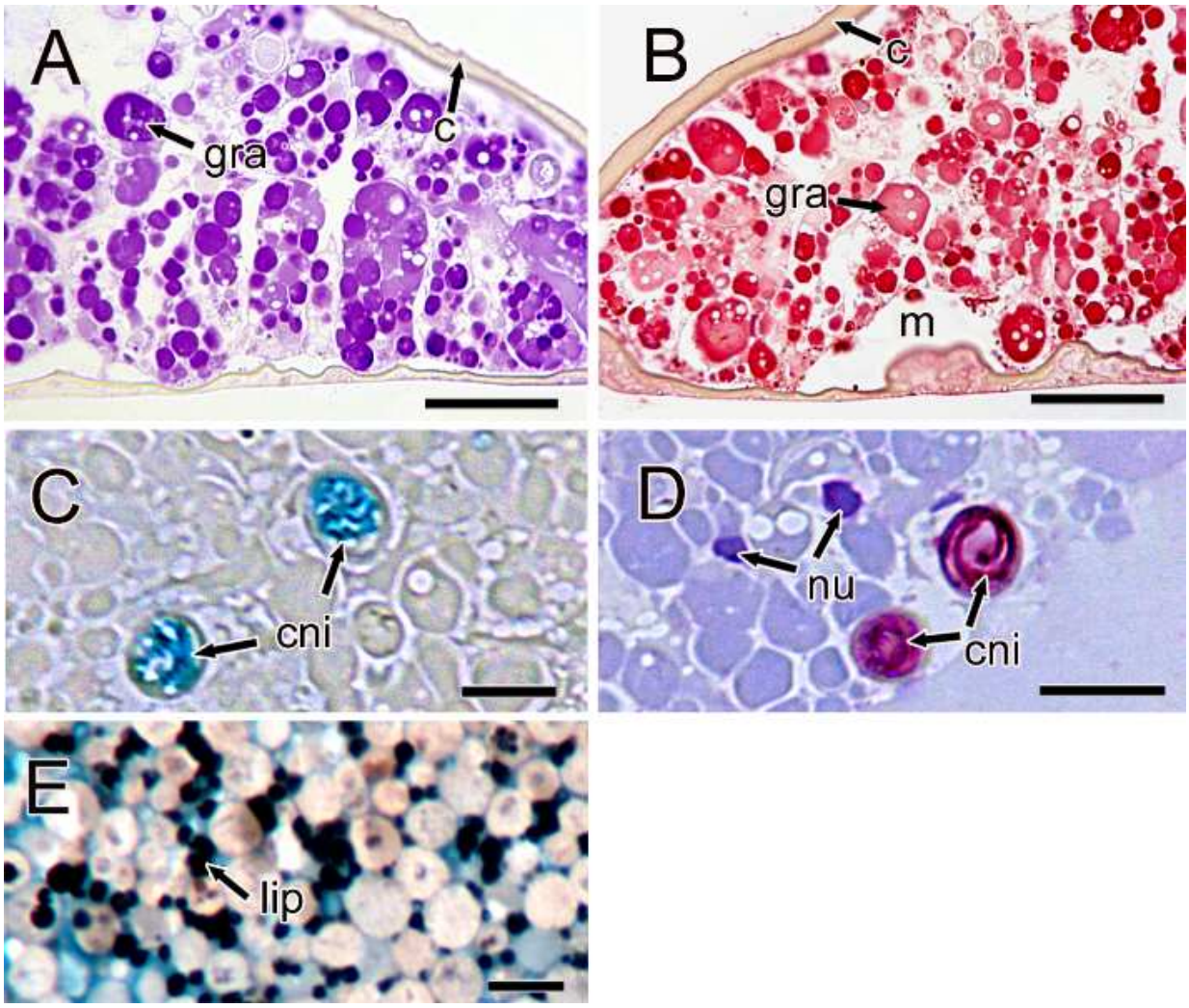

Fig. 9. Photomicrograph of 1-month-old *Aurelia aurita* podocysts showing chemical contents. (A) Carbohydrates stained purple with periodic acid Schiff method. (B) Basic proteins stained red with solochrome cyanin. (C) Acidic mucopolysaccharides stained blue in the cnidoblast with alcian blue. (D) Nuclei containing DNA stained positive with methylgreen. Staining of the cnidoblasts is false. (E) Lipids stained black with Sudan black technique. c, cuticle; m, mucoid layer; gra, granules; cni, cnidoblast; nu, nucleus; lip, lipid. Scale bars=30 µm (A, B) and 10 µm (C, D, E).

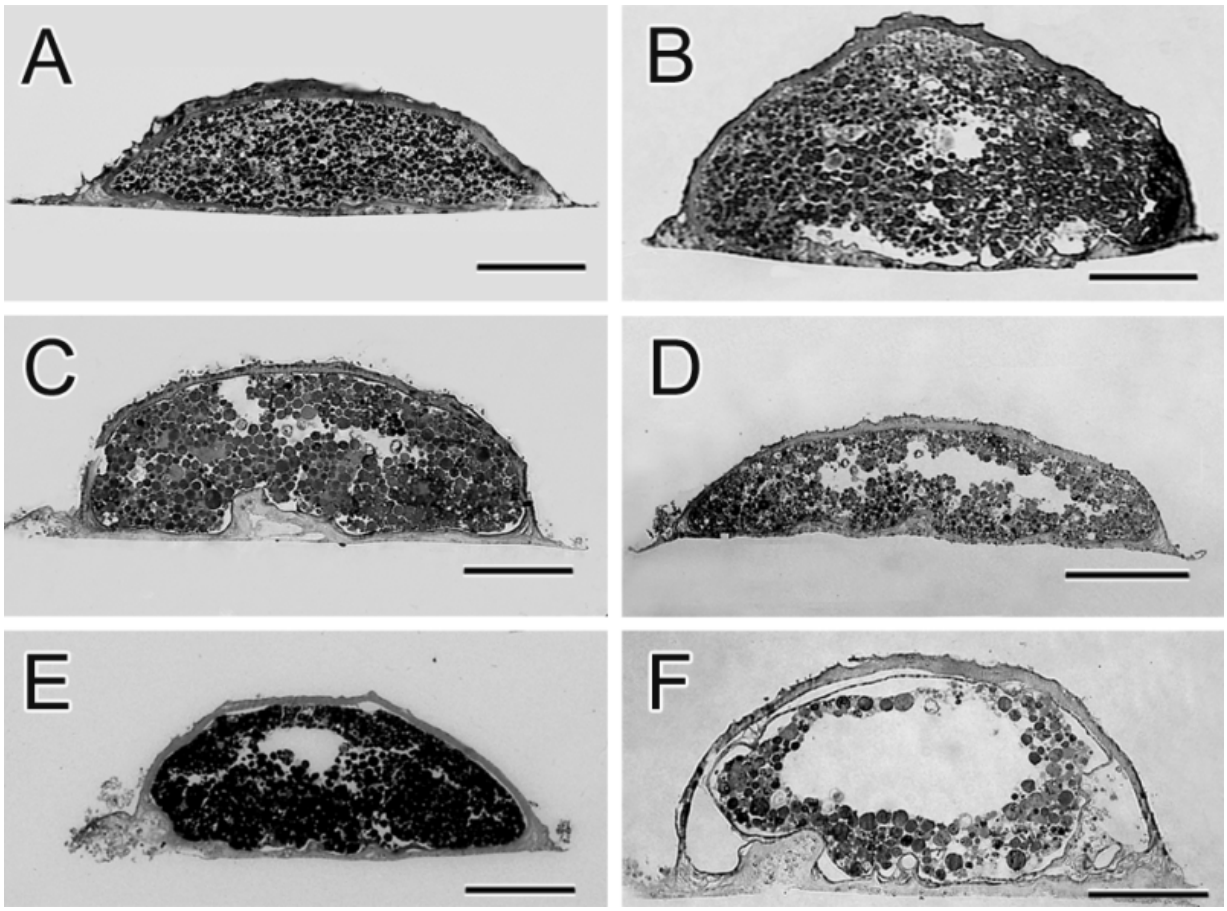

Fig. 10. Photomicrograph of cross sections of *Aurelia aurita* podocysts of different ages. (A) Newly formed. (B) 1-month-old. (C) 3-month-old. (D) 4-month-old. (E) 6-month-old. (F) 12-month-old. Scale bars =100 μm.

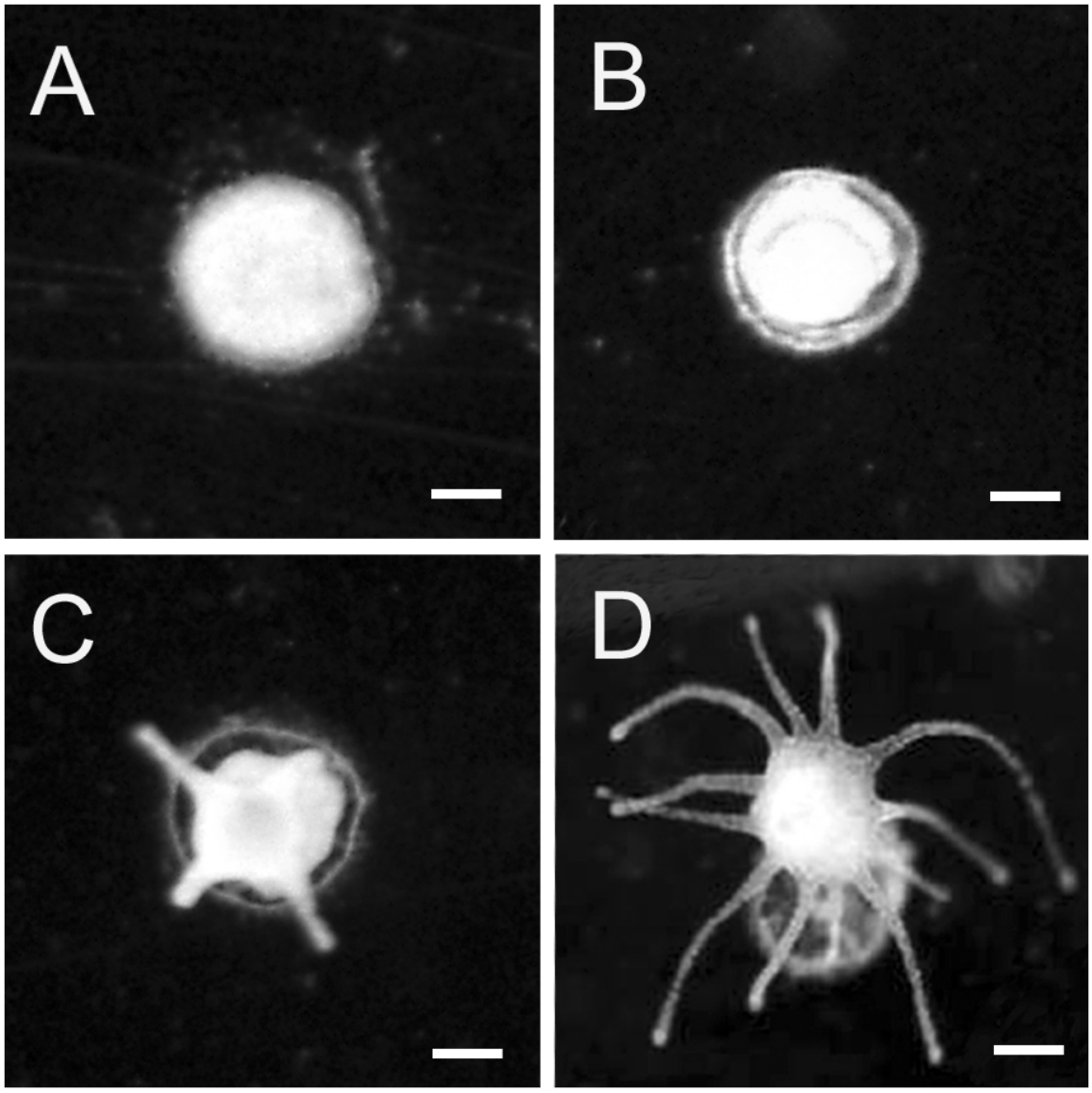

Fig. 11. Top views of the excystment process of 1-month-old *Aurelia aurita* podocysts when they were transferred directly from 28 to 19 °C. (A) A podocyst before the transfer being filled with a whitish-colored cell mass. (B) An excysting podocyst 29 days after the transfer, with the inner cell mass protruding through the emergent hole. (C) A 4-tentacled polyp 3 days after excystment. (D) A 12-tentacled polyp 2 weeks after excystment. Scale bars=100 μm.

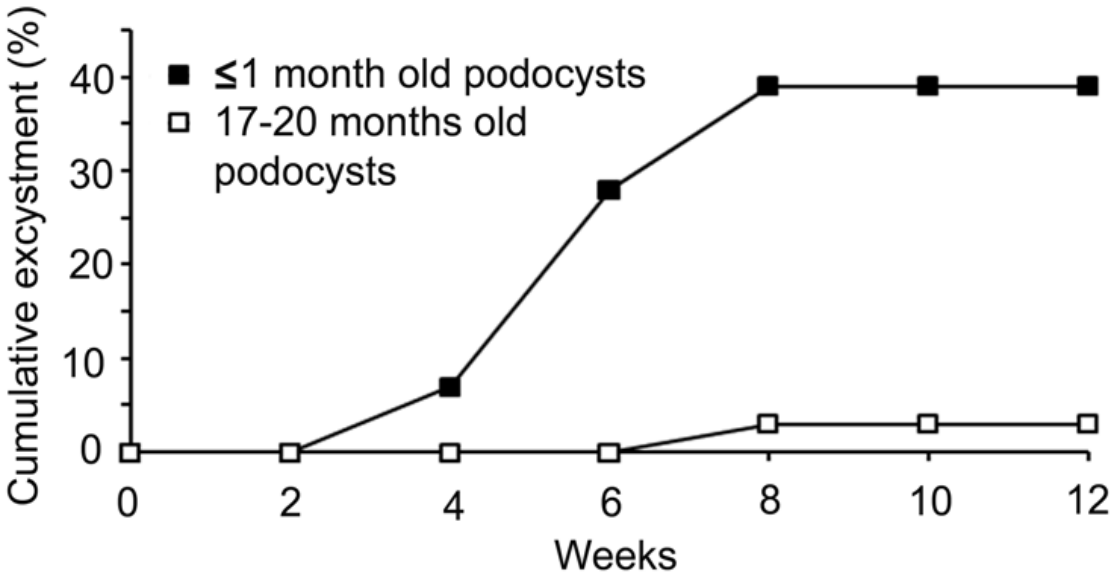

Fig. 12. Cumulative excystment of young (≤1-month-old) and old (17-20-month-old) *Aurelia aurita* podocysts when they were transferred directly from 28 to 19^{o}C.

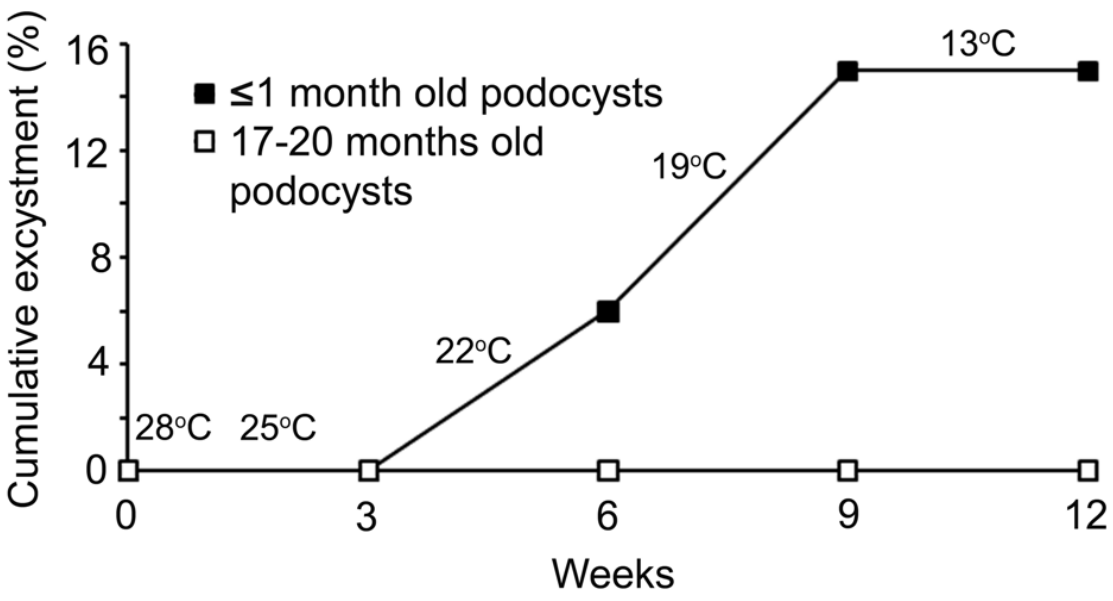

Fig. 13. Cumulative excystment of young (≤1-month-old) and old (17-20-month-old) *Aurelia aurita* podocysts when they were cooled from 28 to 13 °C in a step-wise fashion (i.e. 25, 22, 19, and 13 °C, each for 3 weeks).

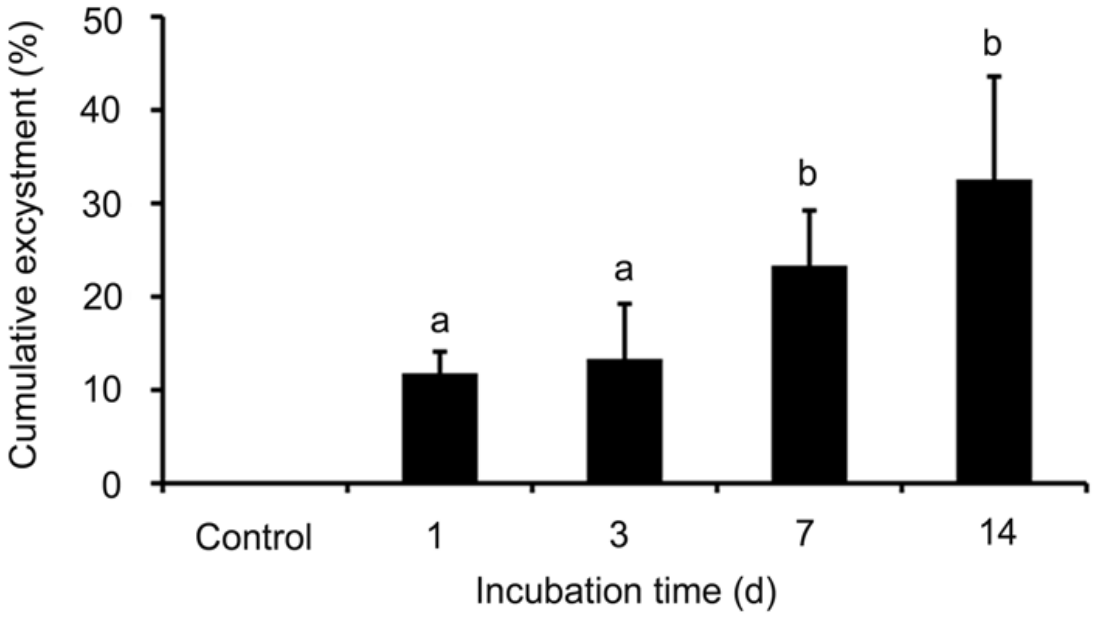

Fig. 14. Cumulative excystment of young (≤1-month-old) *Aurelia aurita* podocysts when they were returned to well-aerated condition from hypoxic condition (0.5 mg O_2 l^{-1}) for 1, 3, 7 and 14 days.

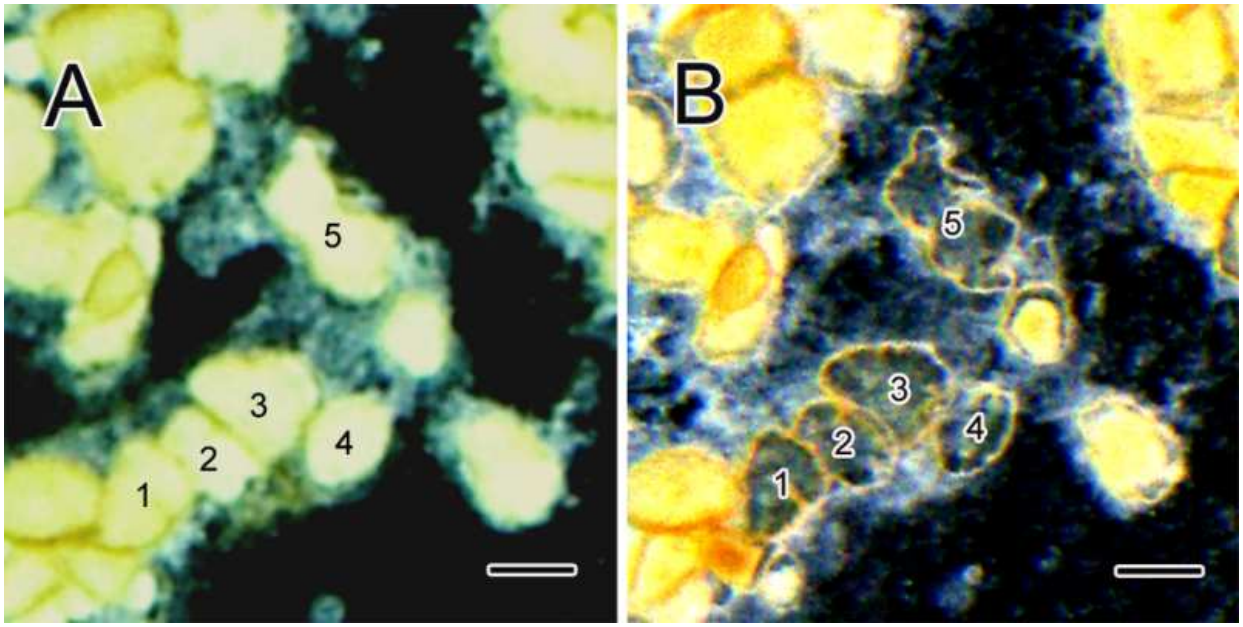

Fig. 15. Photograph of 6-month-old (A) and 25-month-old (B) *Aurelia aurita* podocysts kept at 22 ^{o}C. All the 6-month-old podocysts looked intact, but 5 podocysts (with numerals) were degraded by age of 25-months. Scale bars =300 µm.

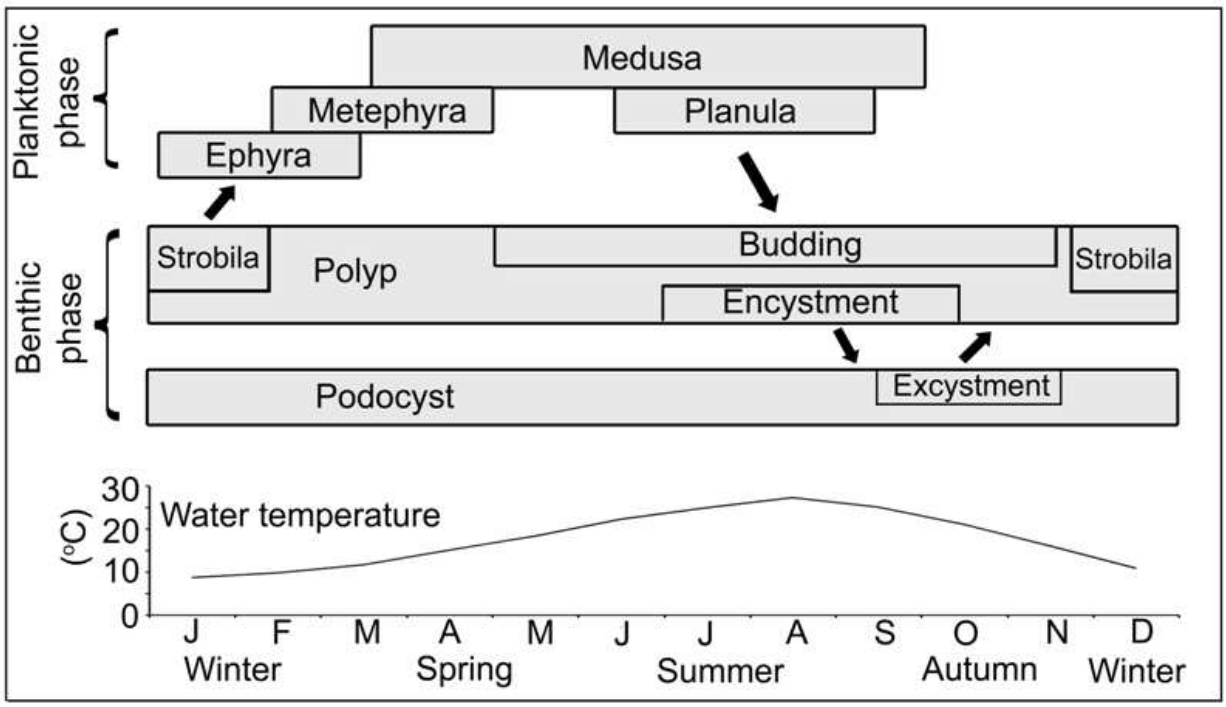

Fig. 16. Schematic representation of the typical seasonal life cycle of *Aurelia aurita* in temperate East Asian coastal waters with special emphasis of the role of podocysts. Seasonal water temperature is the average for the last 30 years in Hiroshima Bay, the Inland Sea of Japan (Hiroshima Prefectural Technology Research Institute,http://www2.ocn.ne.jp/~hfes/current.html).

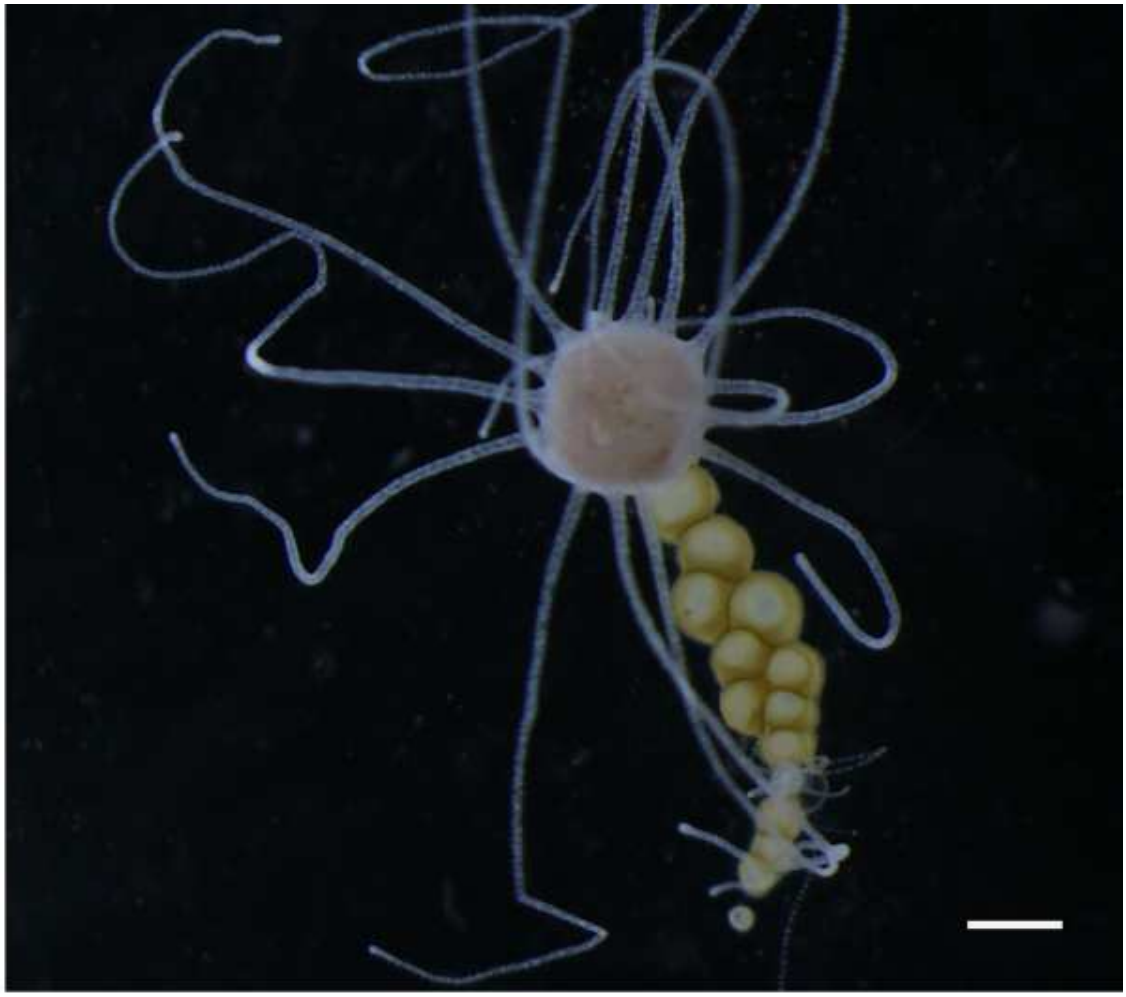

Fig. 17. Formation of *Chrysaora melanaster* podocysts at the base of polyp stalk. Scale bar=1 mm.

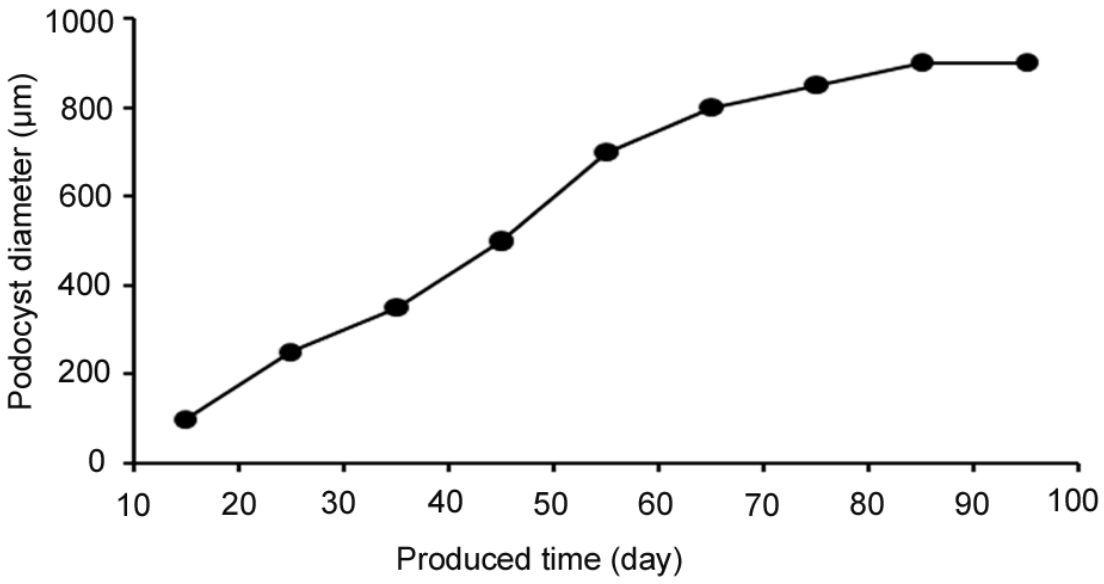

Fig. 18. Increase of diameter of *Chrysaora melanaster* podocysts with time.

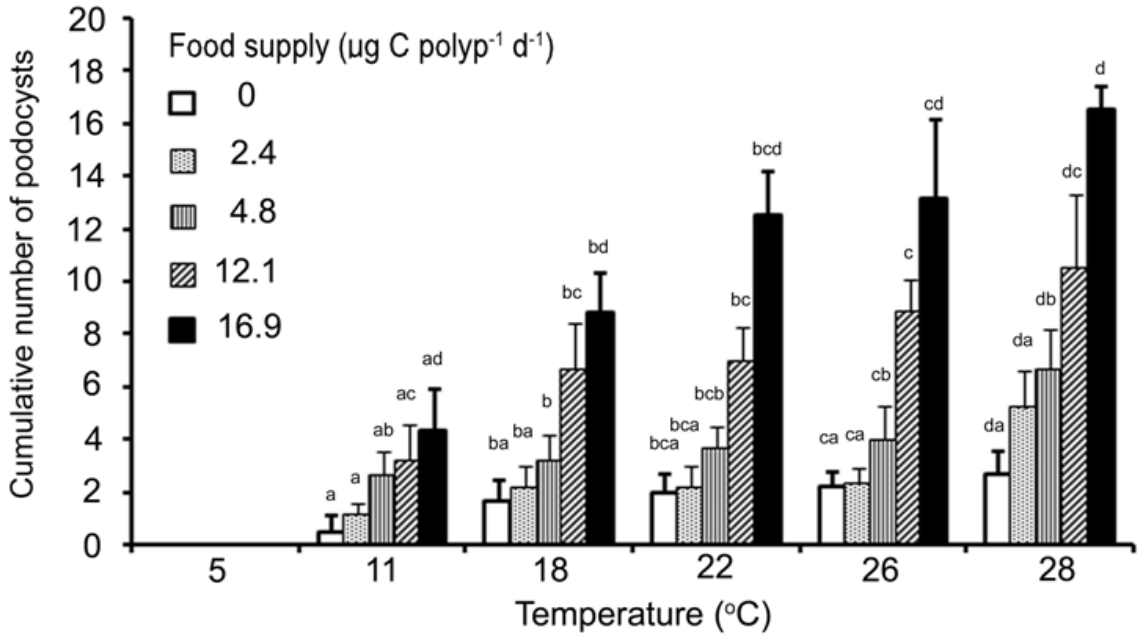

Fig. 19. Cumulative numbers of *Chrysaora melanaster* podocysts produced during 8 weeks under each combination of 6 temperatures and 5 food supplies. Salinity and dissolved oxygen concentration were 32 and ≥5.0 mg O_2 l^{-1}, respectively. Vertical bars denote standard deviations. Means with different letters are significantly different.

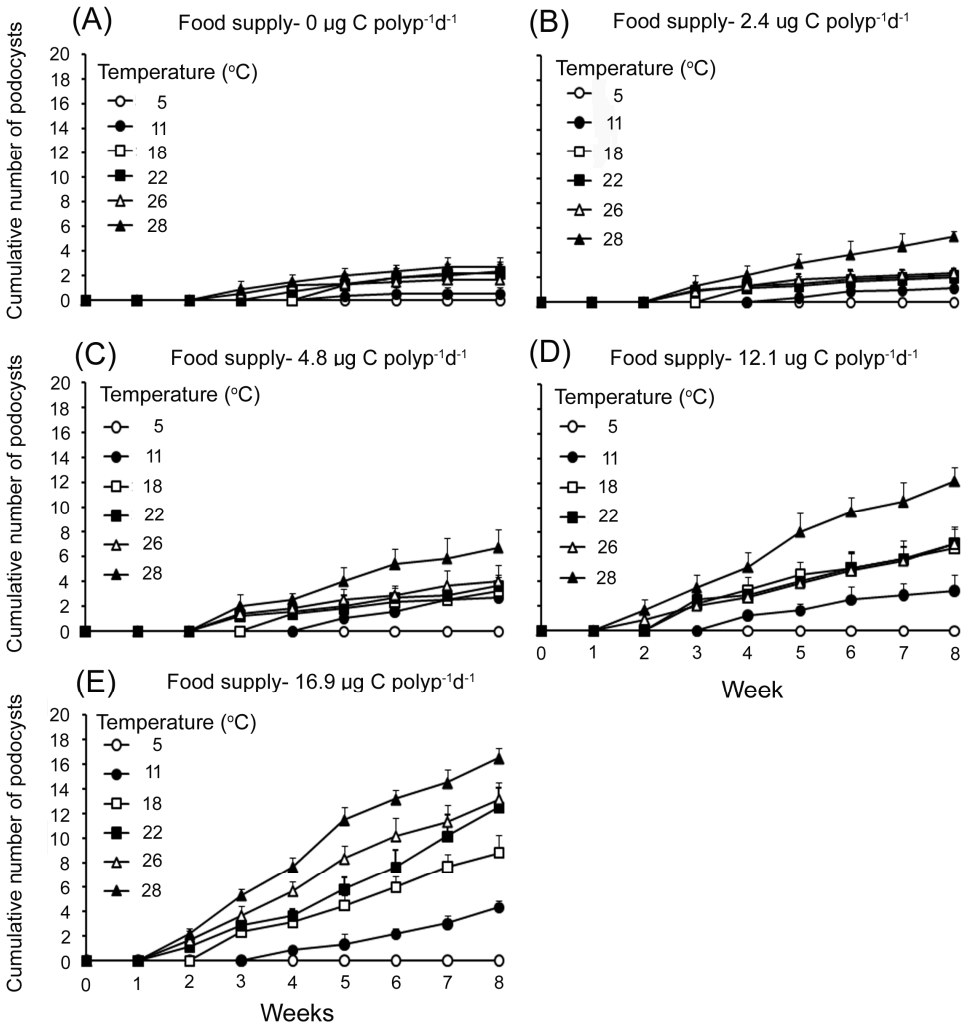

Fig. 20. Cumulative number of *Chrysaora melanaster* podocysts produced at 6 different temperatures at 0 (A), 2.4 (B), 4.8 (C), 12.1 (D) and 16.9 µg C $polyp^{-1}d^{-1}$ (E) during 8 weeks of experiment. Vertical lines denote standard deviations.

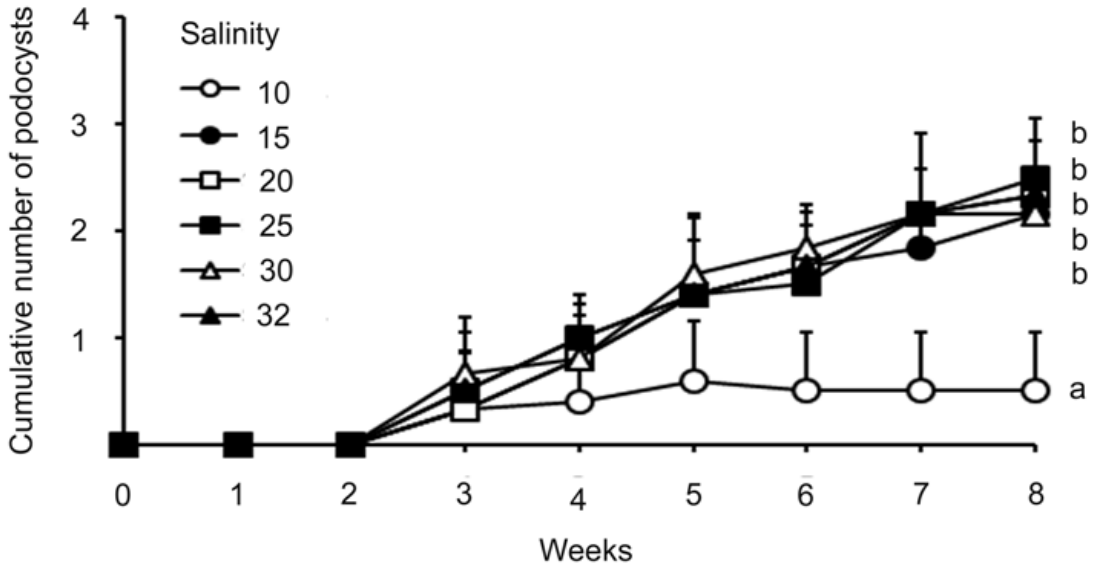

Fig. 21. Cumulative numbers of *Chrysaora melanaster* podocysts produced during 8 weeks at different salinities. Temperature, food supply and dissolved oxygen concentration were 22 ^{o}C, 2.4 µg C polyp^{-1}d^{-1} and ≥5.0 mg O_2 l^{-1}, respectively. Vertical bars denote standard deviations. Means with different letters are significantly different.

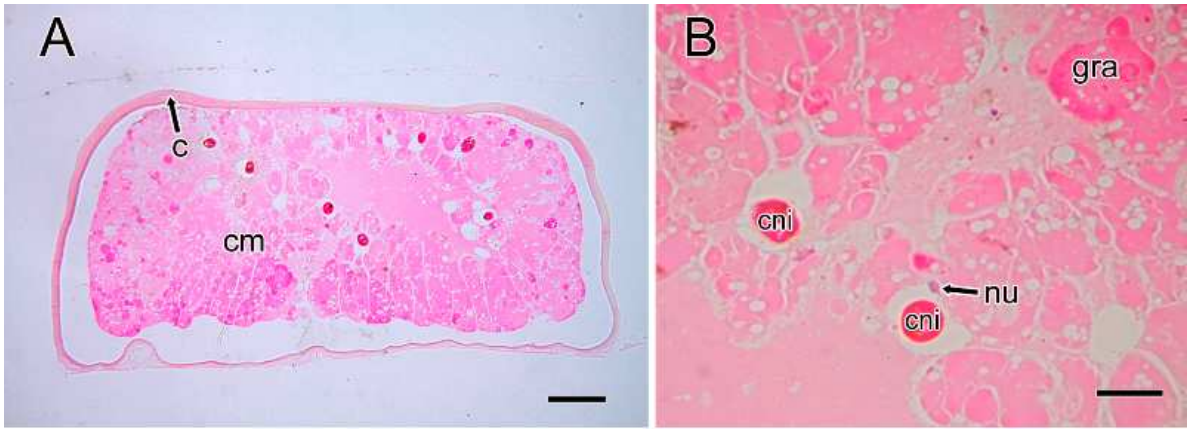

Fig. 22. Photomicrograph of ≤1-month-old *Chrysaora melanaster* podocyst stained with Haematoxylin-eosin method. Cross section (A). Magnified view of nucleus and cnidoblasts in cell mass (B). c, cuticle; cm, cell mass; gra, granules; nu, nucleus; cni, cnidoblast. Scale bars=50 μm (A) and 10 μm (B).

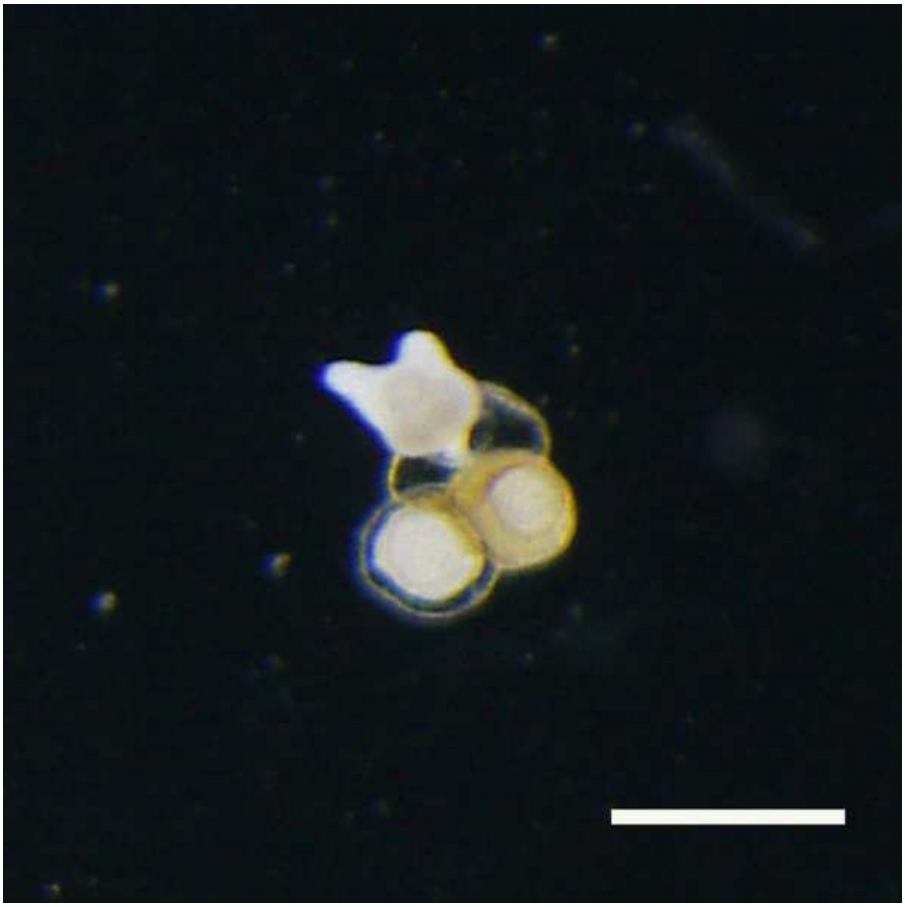

Fig. 23. Top view of the excystment of ≤ 1 month-old *Chrysaora melanaster* podocysts when they were transferred from 28 to 19 ^{o}C. Scale bar=1 mm.

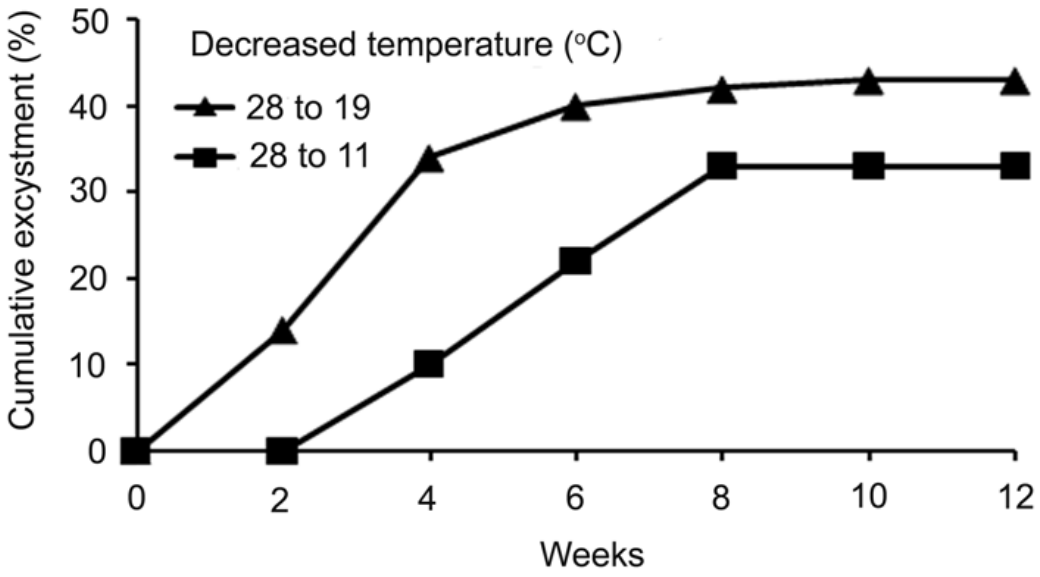

Fig. 24. Cumulative excystment of ≤1-month-old *Chrysaora melanaster* podocysts when they were transferred directly from 28 to 19 °C and 28 to 11 °C.

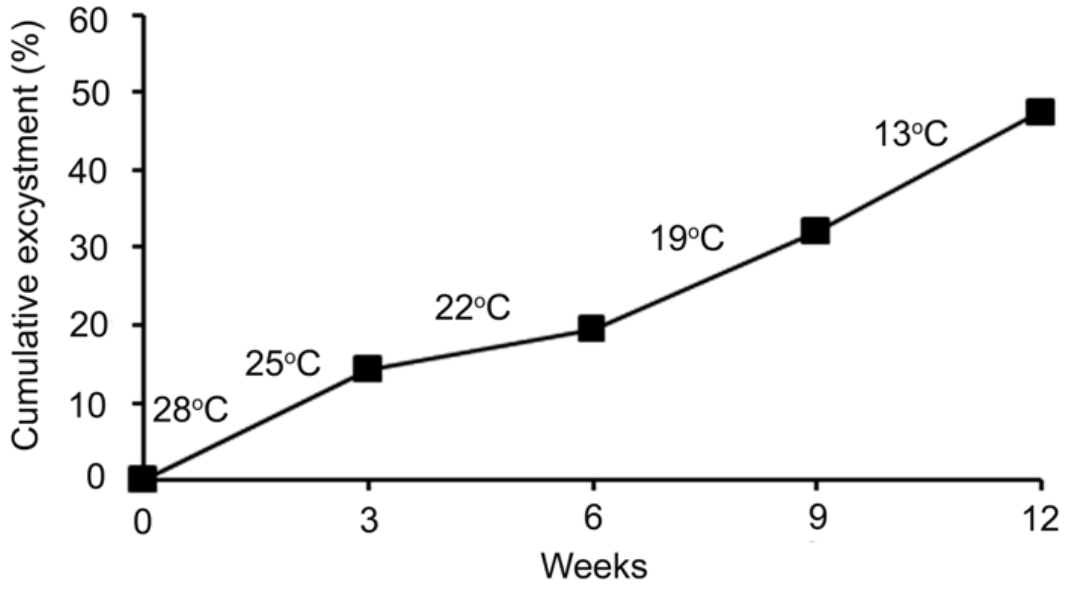

Fig. 25. Cumulative excystment of ≤1-month-old *Chrysaora melanaster* podocysts when they were cooled from 28 to 13 ^{o}C in a step-wise fashion (i.e. 25, 22, 19, and 13 ^{o}C, each for 3 weeks).

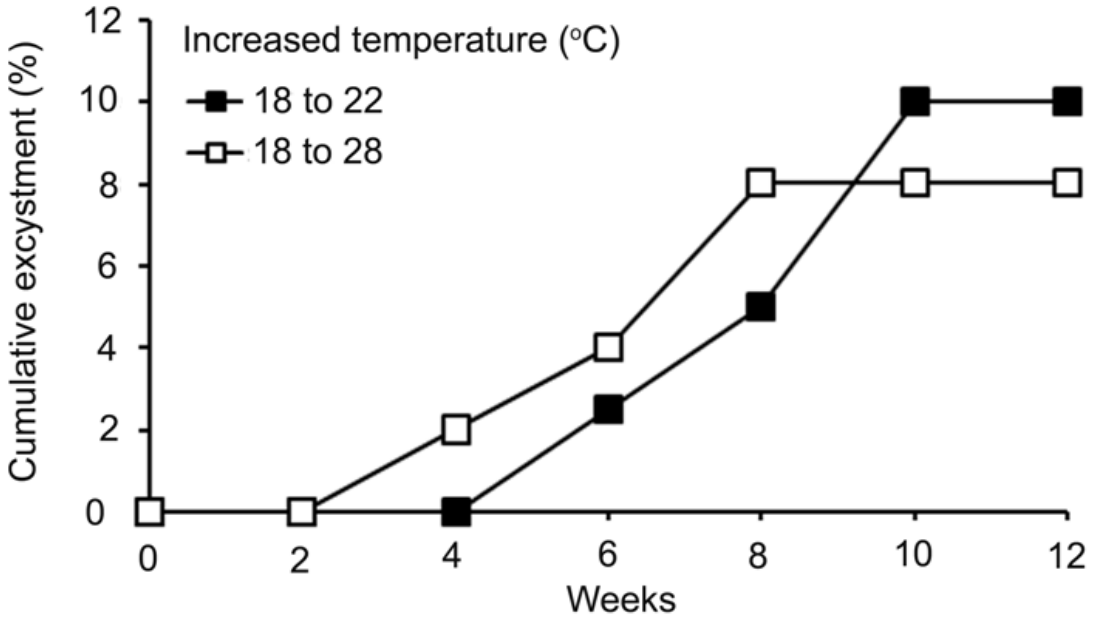

Fig. 26. Cumulative excystment of ≤1-month-old *Chrysaora melanaster* podocysts when they were transferred directly from 18 to 22 °C and 18 to 28 °C.

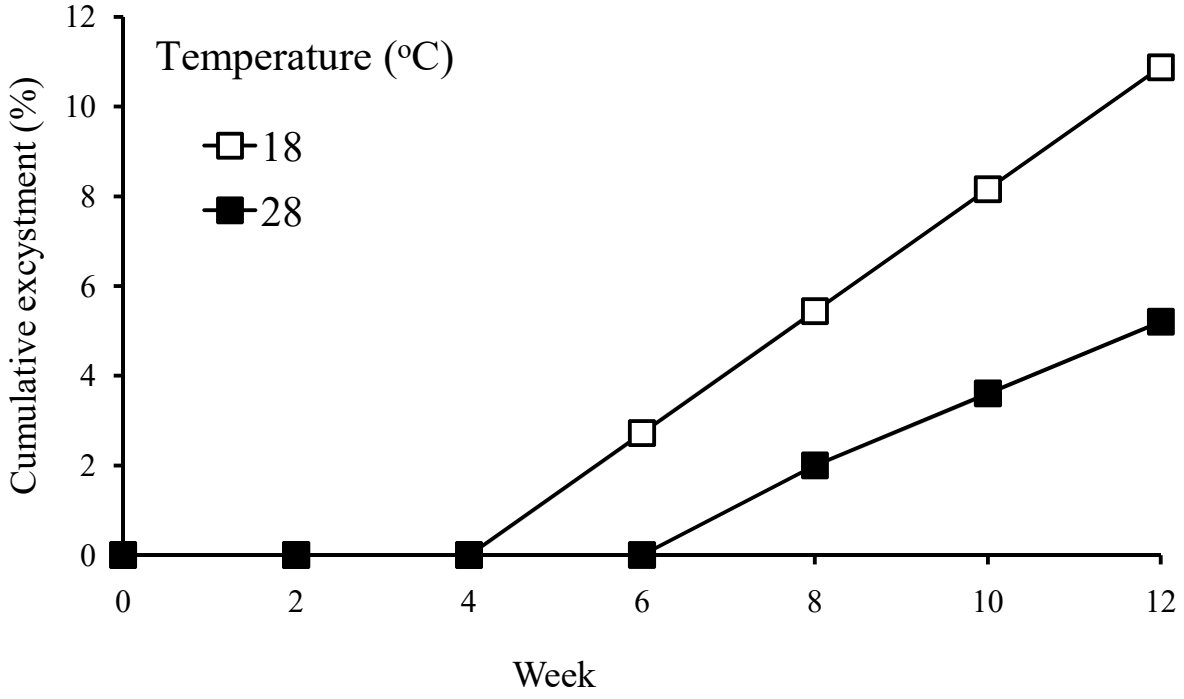

Fig. 27. Cumulative excystment of ≤1-month-old *Chrysaora melanaster* podocysts kept at constant temperatures of 18 and 28 °C.

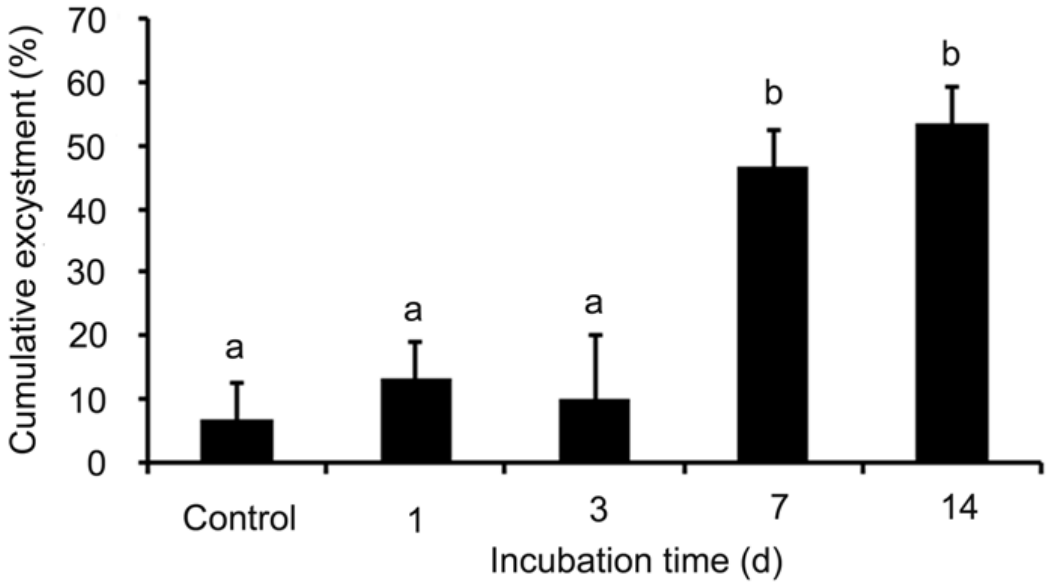

Fig. 28. Cumulative excystment of ≤1-month-old *Chrysaora melanaster* podocysts when they were returned to well-aerated condition from hypoxic condition (0.5 mg O_2 l^{-1}) for 1, 3, 7 and 14 day.

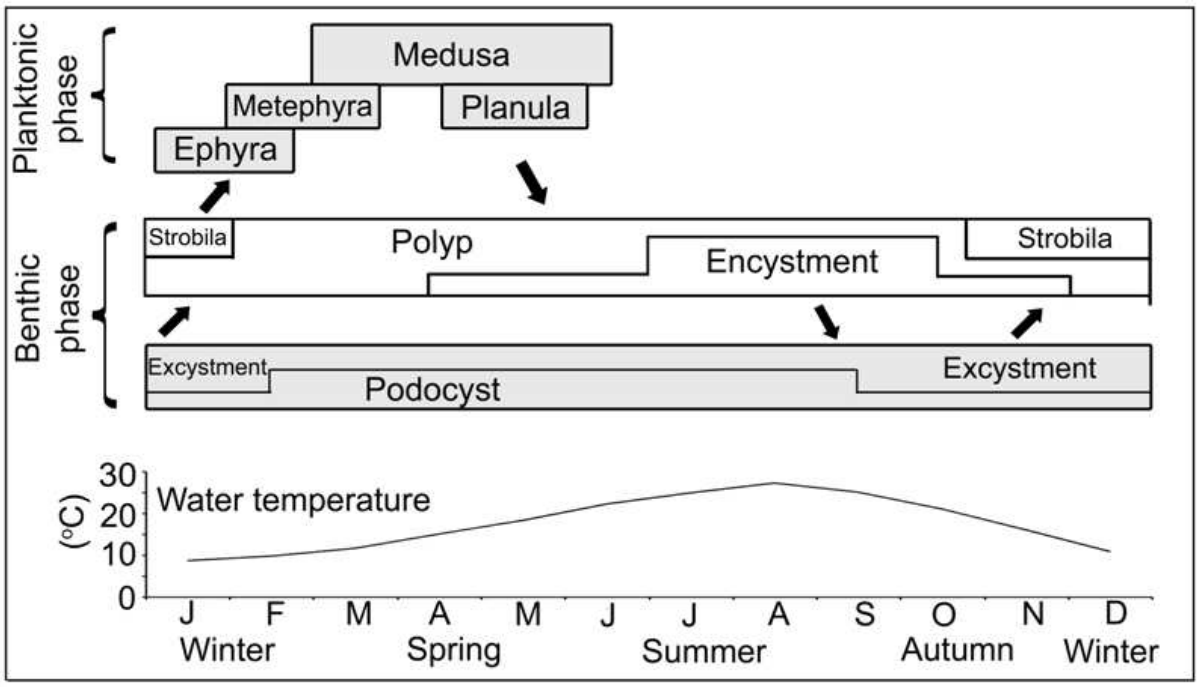

Fig. 29. Schematic representation of the typical seasonal life cycle of *Chrysaora melanaster* in temperate East Asian coastal waters with special emphasis of the role of podocysts. Seasonal water temperature is the average for the last 30 years in Hiroshima Bay, the Inland Sea of Japan (Hiroshima Prefectural Technology Research Institute, http://www2.ocn.ne.jp/~hfes/current.html).

Fig. 30. Formation of *Cyanea nozakii* podocysts at the base of polyp stalk at 22 ^{o}C for 3 months. Scale bar = 500 μm.

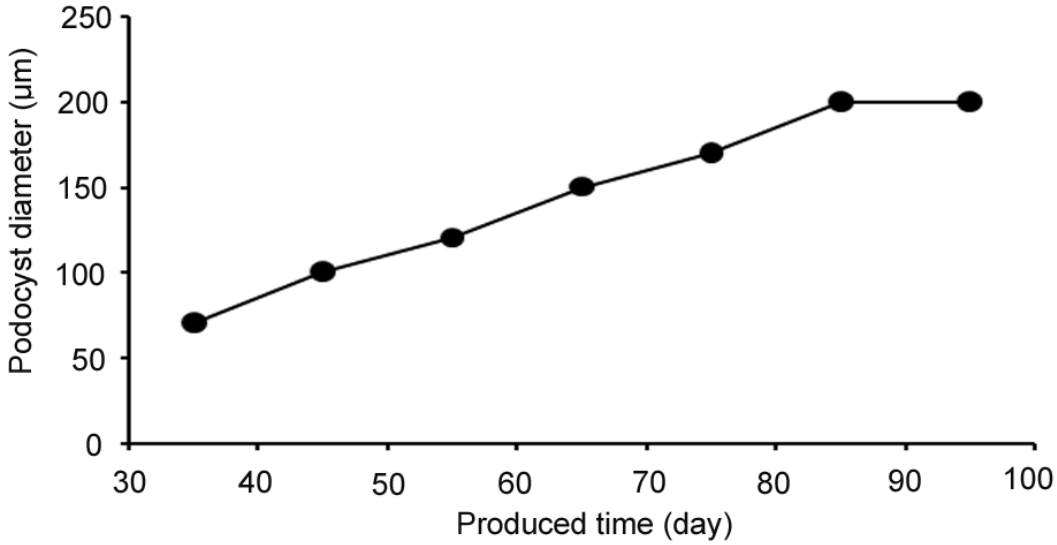

Fig. 31. Increase of diameter of *Cyanea nozakii* podocysts with time.

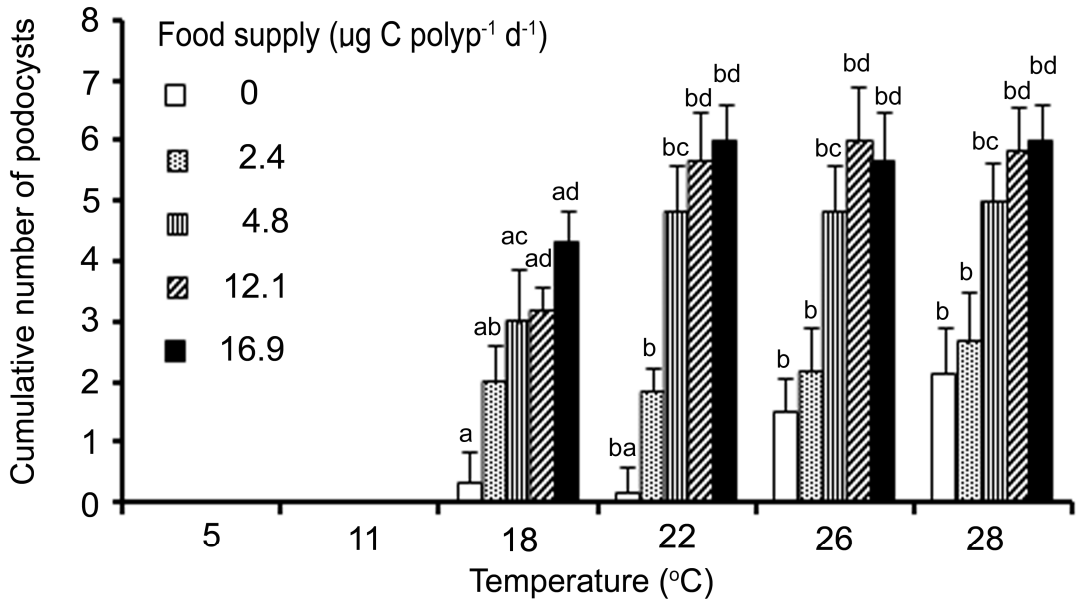

Fig. 32. Cumulative numbers of *Cyanea nozakii* podocysts produced during 8 weeks under each combination of 6 temperatures and 5 food supplies. Salinity and dissolved oxygen concentration were 32 and ≥5.0 mg O_2 l^{-1}, respectively. Vertical bars denote standard deviations. Means with different letters are significantly different.

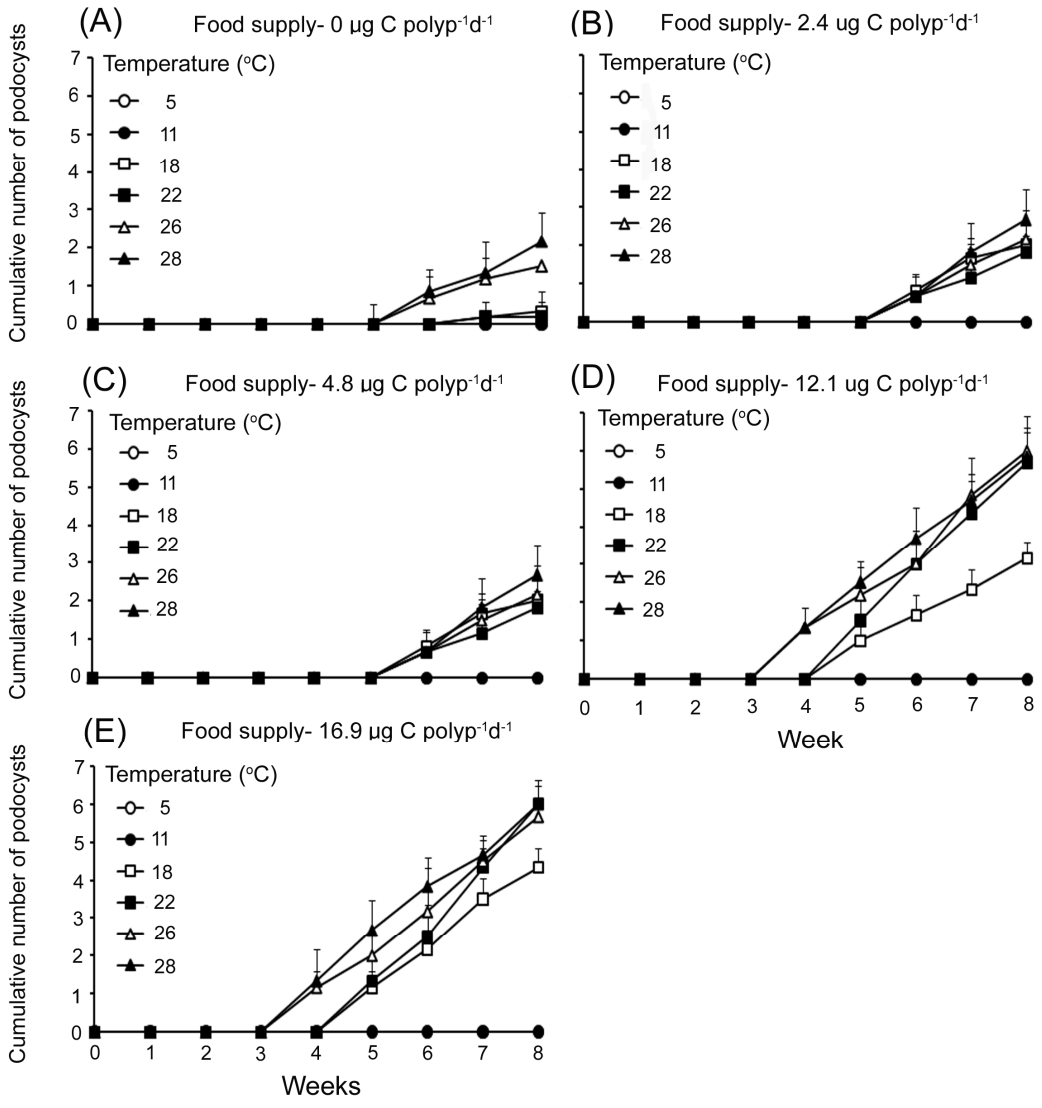

Fig. 33. Cumulative number of *Cyanea nozakii* podocysts produced in 6 different temperatures at 0 (A), 2.4 (B), 4.8 (C), 12.1 (D) and 16.9 µg C $polyp^{-1}d^{-1}$ (E) during 8 weeks of experiment. Vertical lines denote standard deviations.

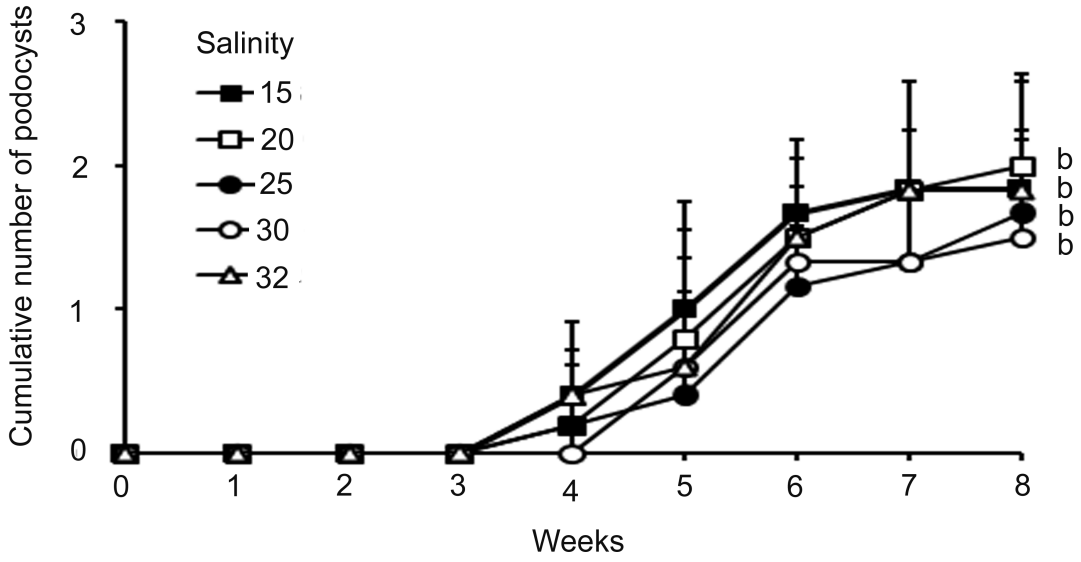

Fig. 34. Cumulative numbers of *Cyanea nozakii* podocysts produced during 8 weeks at different salinities. Temperature, food supply and dissolved oxygen concentration were 22 ^{o}C, 2.4 µg C polyp^{-1}d^{-1} and ≥5.0 mg O_2 l^{-1}, respectively. Vertical bars denote standard deviations. Means with different letters are significantly different.

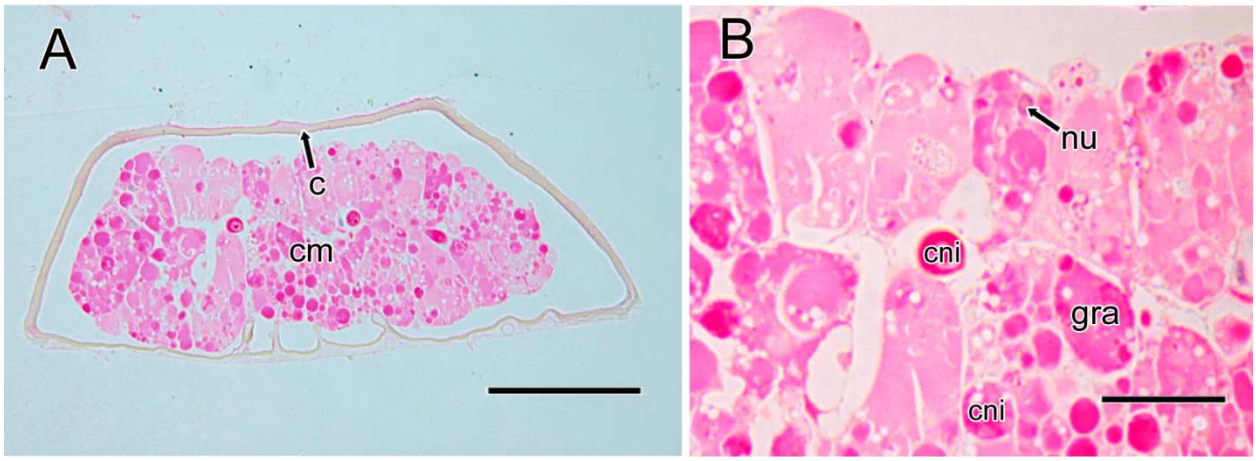

Fig. 35. Photomicrograph of ≤1-month-old *Cyanea nozakii* podocyst stained with Haematoxylin-eosin method. Cross section (A). Magnified view of nucleus and cnidoblasts in cell mass (B). c, cuticle; cm, cell mass; gra, granules; nu, nucleus; cni, cnidoblast. Scale bars=50 μm (A) and 10 μm (B).

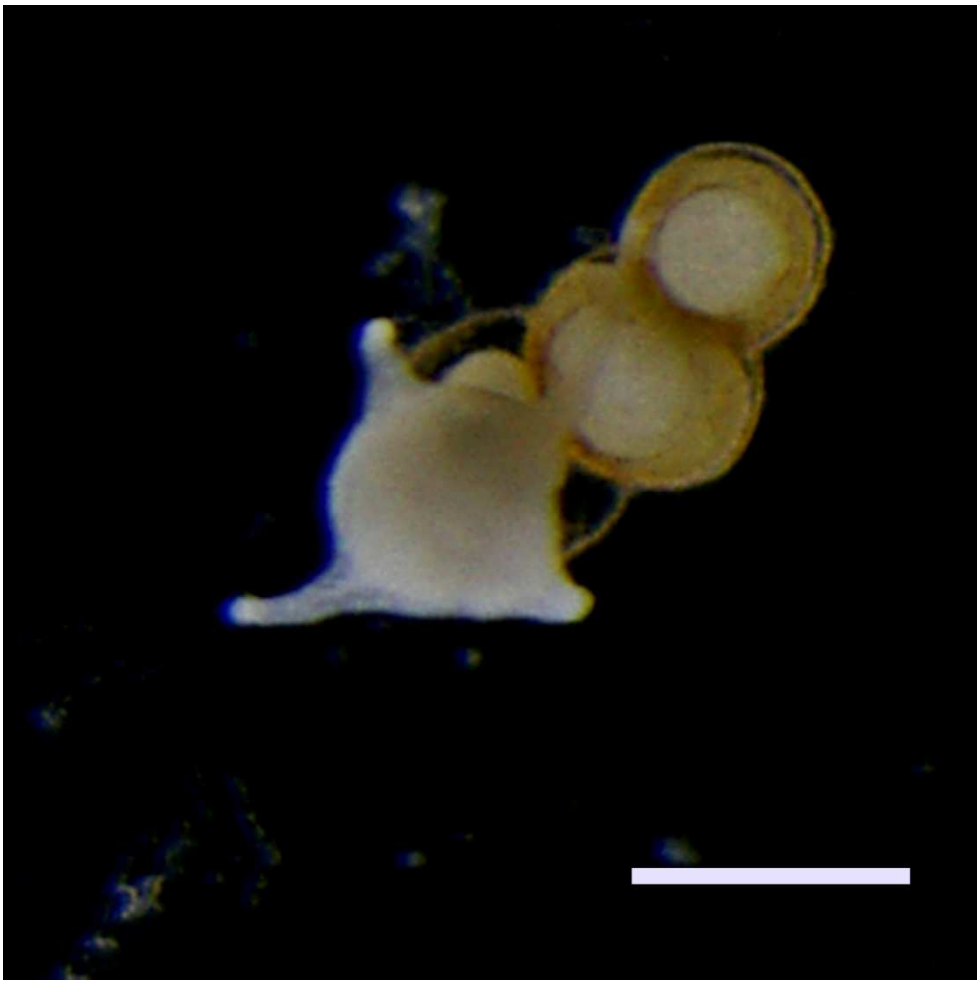

Fig. 36. Top view of the excystment of ≤ 1month-old *Cyanea nozakii* podocysts when they were transferred from 28 to 19 ^{o}C). Scale bar=200 µm.

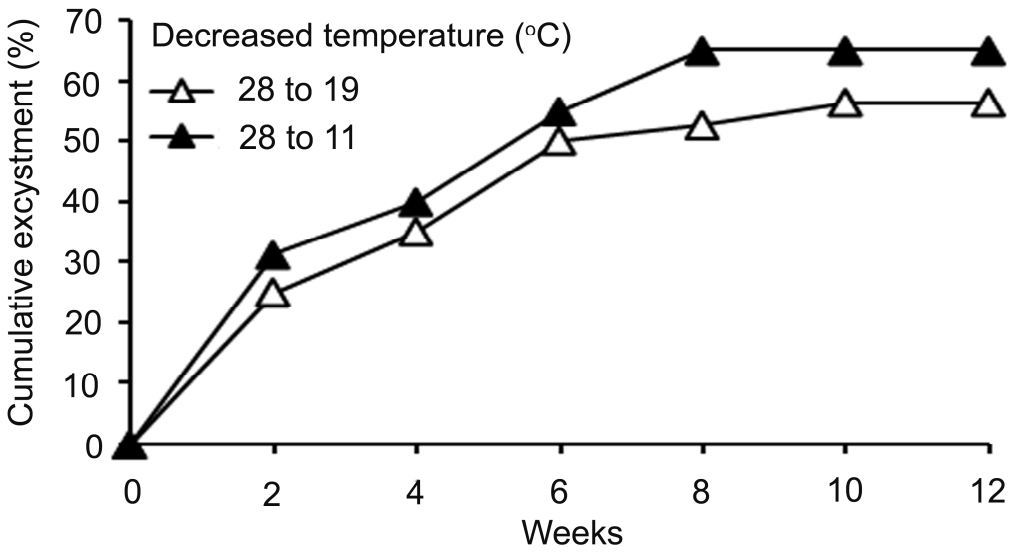

Fig. 37. Cumulative excystment of ≤1-month-old *Cyanea nozakii* podocysts when they were transferred directly from 28 to 19 °C and 28 to 11 °C.

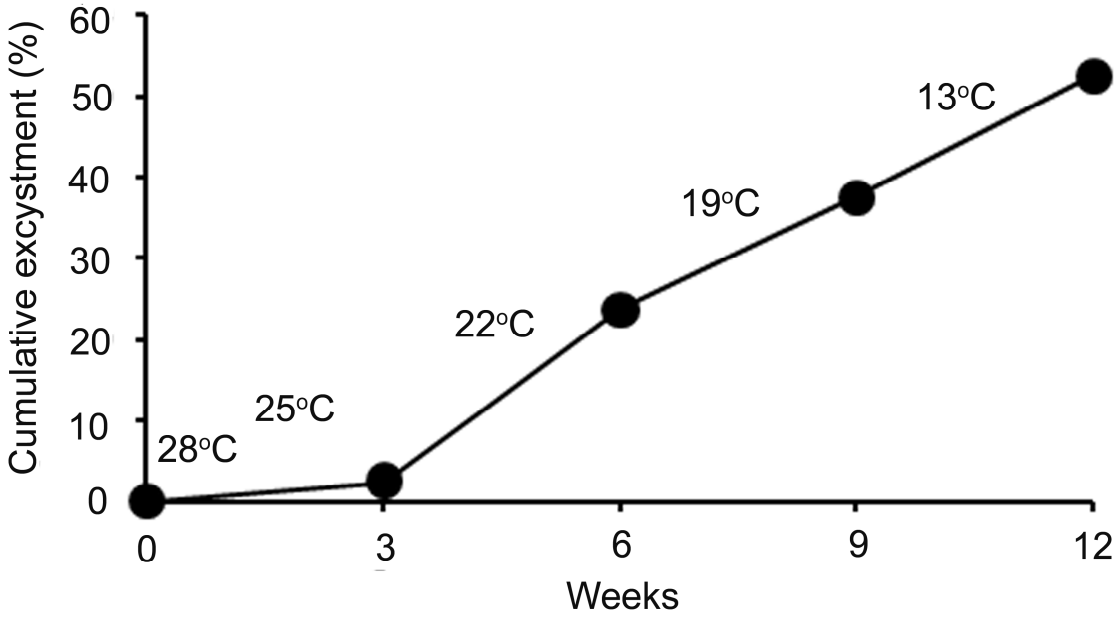

Fig. 38. Cumulative excystment of ≤1-month-old *Cyanea nozakii* podocysts when they were cooled from 28 to 13 °C in a step-wise fashion (i.e. 25, 22, 19, and 13 °C, each for 3 weeks).

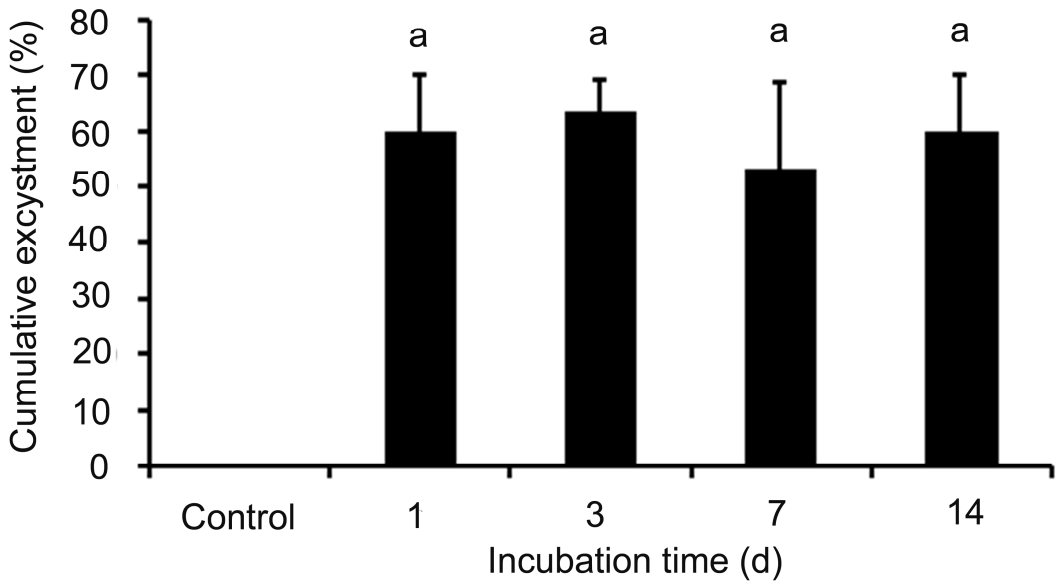

Fig. 39. Cumulative excystment of ≤1-month-old *Cyanea nozakii* podocysts when they were returned to well-aerated condition from hypoxic condition (0.5 mg O_2 l^{-1}) for 1, 3, 7 and 14 days.

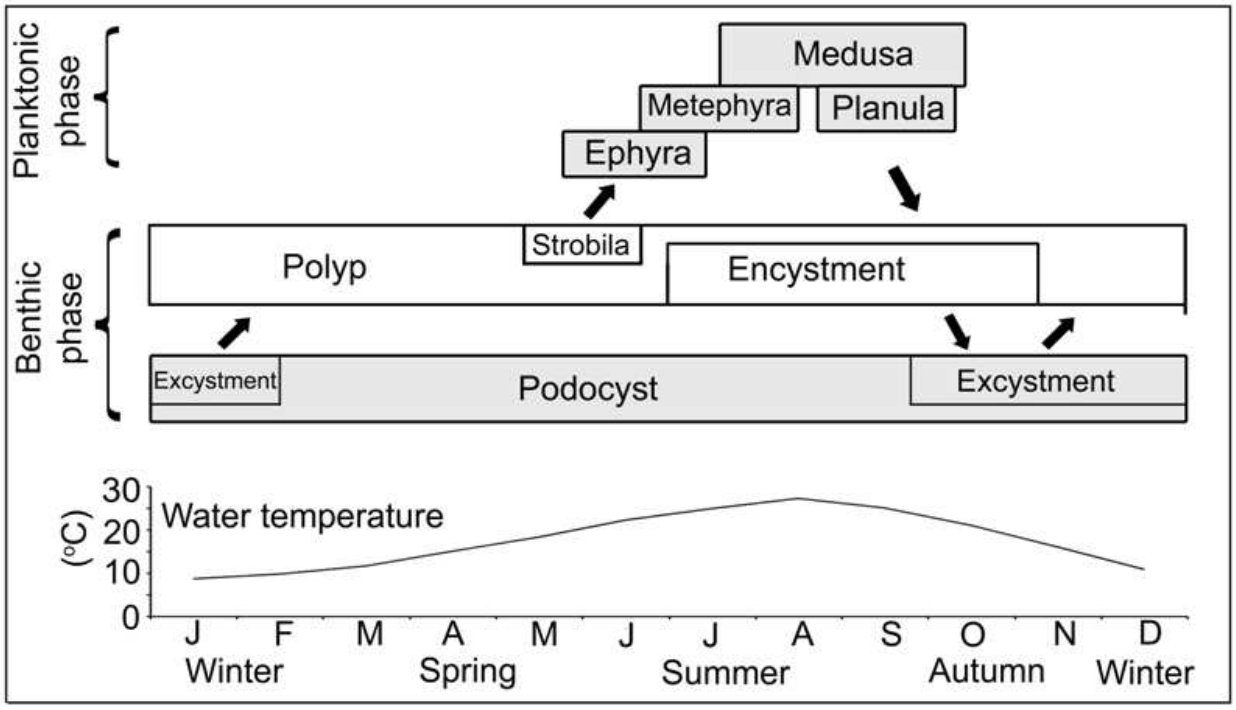

Fig. 40. Schematic representation of the typical seasonal life cycle of *Cyanea nozakii* in temperate East Asian coastal waters with special emphasis of the role of podocysts. Seasonal water temperature is the average for the last 30 years in Hiroshima Bay, the Inland Sea of Japan (Hiroshima Prefectural Technology Research Institute,http://www2.ocn.ne.jp/~hfes/current.html).

Table. 1 Podocysts production of *Aurelia aurita, Chrysaora melanaster* and *Cyanea nozakii* in various combinations of temperatures and food supplies during 8 weeks of experiment. Salinity and dissolved oxygen concentration were 32 and ≥5.0 mg O_2 l^{-1}, respectively. SD is given in parenthesis.

Species	Tempe-rature (°C)	Food supply (µg C $polyp^{-1}$ d^{-1})					Statistics
		0	2.4	4.8	12.1	16.9	
Aurelia aurita	5	0	0	0	0	0	*Temperature* ($F_{5, 150}$=147.230, p<0.001)
	11	0.3(0.5)	0.2 (0.4)	0	0	0	
	18	2.7 (0.5)	2.0 (0.6)	0.8 (0.4)	0	0	*Food* ($F_{4, 150}$=257.472, p<0.001)
	22	5.0 (1.1)	3.2 (0.8)	1.8 (0.8)	0	0	
	26	5.8 (1.0)	4.0 (0.6)	2.7 (0.8)	0	0	*Food*×*Tem* ($F_{20, 150}$=30.183, p<0.001)
	28	6.0 (1.1)	5.2 (1.2)	3.0 (0.9)	0	0	
Chrysaora melanaster	5	0	0	0	0	0	*Temperature* ($F_{5, 150}$=177.237, p<0.001)
	11	0.5 (0.6)	1.2 (0.4)	2.7 (0.8)	3.2 (1.3)	4.3 (1.5)	
	18	2.3 (0.8)	2.2 (0.8)	3.2 (1.0)	6.7 (1.6)	8.8 (1.5)	*Food* ($F_{4, 150}$=215.497, p<0.001)
	22	2.2 (0.6)	2.2 (0.8)	3.7 (0.8)	7 (1.3)	12.5 (1.6)	
	26	1.2 (0.5)	2.3 (0.5)	4.0 (1.3)	7 (1.2)	13.2 (2.9)	*Food*×*Tem* ($F_{20, 150}$=20.535, p<0.001
	28	2.7 (0.8)	5.2 (1.3)	6.7 (1.5)	12.1 (2.7)	16.5 (0.8)	
Cyanea nozakii	5	0	0	0	0	0	*Temperature* ($F_{5, 150}$=366.674, p<0.001)
	11	0	0	0	0	0	
	18	0.3 (0.5)	2.0 (0.6)	3.0 (0.9)	3.2 (0.4)	4.3 (0.5)	*Food* ($F_{4, 150}$=185.529, p<0.001)
	22	0.2 (0.4)	1.8 (0.4)	4.8 (0.8)	5.6 (1.2)	6.0 (0.6)	
	26	1.5 (0.5)	2.2 (0.8)	4.8 (0.8)	6.0 (0.9)	5.7 (0.8)	*Food*×*Tem* ($F_{20, 150}$=22.464, p<0.001
	28	2.2 (0.8)	2.7 (0.8)	5.3 (0.8)	5.8 (0.8)	6.0 (0.6)	

Table 2. Effect of salinity on podocysts production of *Aurelia aurita, Chrysaora melanaster* and *Cyanea nozakii* during 8 weeks of experiment. Temperature, food supply and dissolved oxygen concentration were 22 ^{o}C, 2.4 µg C polyp^{-1}d^{-1} and ≥5.0 mg O_2 l^{-1}, respectively. SD is given in parenthesis.

Species	Salinity	Numbers of podocysts produced	Statistics
Aurelia aurita	5	0 (0)	($F_{4,25}$=2.591, p<0.061)
	10	0 (0)	
	15	2.3 (0.5)	
	20	2.8 (0.7)	
	25	3.2 (0.7)	
	30	3.2 (0.4)	
	32	3.3 (0.5)	
Chrysaora melanaster	5	0 (0)	($F_{5,30}$=13.636, p<0.001)
	10	0.5 (0.5)	
	15	2.2 (0.4)	
	20	2.3 (0.5)	
	25	2.5 (0.5)	
	30	2.2 (0.4)	
	32	2.3 (0.5)	
Cyanea nozakii	5	0 (0)	($F_{4,\ 25}$=0.637, p=0.641)
	10	0 (0)	
	15	1.8 (0.4)	
	20	2.0 (0.6)	
	25	1.7 (0.5)	
	30	1.5 (0.5)	
	32	1.8 (0.7)	

Table 3. Effect of dissolved oxygen concentration on excystment of podocysts of *Aurelia aurita, Chrysaora melanaster* and *Cyanea nozakii*. See text for detail.

Species	Numbers of podocysts examined	Experimental conditions		Excystment (%)	Statistics
		DO (mg O_2 l^{-1})	Duration (Days)		
Aurelia aurita	30	0.5	1	11.8	
	30	0.5	3	13.3	($F_{4,10}$=11.948, p<0.007)
	30	0.5	7	23.3	
	30	0.5	14	32.5	
(Control)	30	>5.0	28	0	
Chrysaora melanaster	30	0.5	1	13.3	
	30	0.5	3	10.0	($F_{4,10}$=31.571, p<0.000)
	30	0.5	7	46.7	
	30	0.5	14	53.3	
(Control)	30	>5.0	28	6.7	
Cyanea nozakii	30	0.5	1	60.0	
	30	0.5	3	63.3	($F_{3,8}$=0.425, p>0.725)
	30	0.5	7	53.3	
	30	0.5	14	60.0	
(Control)	30	>5.0	28	0	

Table 4. Examination of maximum longevity of *Aurelia aurita* podocysts at 18, 22 and 28 ^{o}C.

Duration (Year)	Number of podocysts examined	Temperature (^{o}C)		
		18	22	28
		Alive/Dead	Alive/Dead	Alive/Dead
1.0	100	A	A	A
1.2	97	A	A	A
1.9	115	A	A	A
2.5	108	A	A	D
2.8	75	A	A	D
3.1	95	A	A	D
3.7	108	D	D	D

Table 5. Comparison of asexual reproduction patterns of polyps among *Aurelia aurita, Chrysaora melanaster, Cyanea nozakii* and *Nemopilema nomurai.*

Species	Modes of asexual reproduction	Podocyst			Budding rate (polyp^{-1} week^{-1})	Type of strobila	Order of reproductive potential
		Maximum production for 6 months	Maximum longevity	Maximum production rate (polyp^{-1} week^{-1})			
Aurelia aurita	Budding Fission Podocyst	6 (for 2 months)	3.2 years	0.75	8.1	Poly-disc	1
Chrysaora melanaster	Podocyst	17	< 1 year	2.1	No	Poly-disc	2
Cyanea nozakii	Podocyst Planulocyst	6	> 1 year	0.75	No	Mono-disc	3
Nemopilema nomurai	Podocyst	16	> 5 years	0.4	No	Poly-disc	4