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Taxonomic Composition and Seasonal Distribution Changes of Pelagic Tunicates in the Waters Off Nuclear Power Plants in Northern Taiwan in Relation to Environmental Conditions

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Pietro Franco, HongJu Chen, and Jiang Shiou Hwang (2016) The ecological importance of pelagic tunicates and their role in marine ecosystems are receiving increasing attention, especially with respect of their role in the carbon flow through the planktonic food web and their fundamental influence on the microbial food web. Nuclear Power Plants I and II are located in most populated northern Taiwan. Effects of the discharge of the cooling water from nuclear power plants have drawn great attention since 1977. No studies on pelagic tunicates were carried out in the area. Taxonomic composition, distribution, abundance and seasonality of pelagic tunicates belonging to the two taxonomic classes Larvacea and Thaliacea were studied and their relationship with environmental conditions were analyzed from 21 stations in the waters adjacent to 2 nuclear power plants in northern Taiwan. A total of nine species were identified: the appendicularians *Oikopleura dioica*, *O. caphocera*, *O. rufuscens*, *O. longicauda*, *Fritillaria aberrans* and the thaliaceans *Doliolum denticulatum*, *Thalia democratica orientalis*, *Dolioletta gegenbauri* and *Thetys vagina*. There is a strong seasonality in the area. In fact, the highest abundances were measured during the summer season, whereas low abundances prevailed in the rest of the year. No relationship was found between tunicate abundance and surface temperature and salinity, likely due to slight changes of the two environmental parameters of the studied area within each season. The hypothesis regarding a direct effect of the discharged cooling waters on the organisms was consequently discarded. The abundances of larvaceans and thaliaceans were significantly influenced by the concentration of chl-*a*, among the four seasons as well as within all the stations for each season.There is a strong seasonality in the area concerning the distribution and abundance values of the nine species of pelagic tunicates in the area.The presence of the organisms is more influenced by the availability of food during the four seasons rather than by the effects of the discharged waters from the two nuclear plants. Further research needs to describe inter-annual changes in seasonality of pelagic tunicates in this area.

Key words: Nuclear power plant, Pelagic tunicate, Species composition, Distribution, Environmental condition.

BACKGROUND

Pelagic tunicates are distributed worldwide (Deibel 1998), mostly on continental shelves or coastal waters (Wiebe et al. 1979) and even estuaries (Ritz et al. 2003). The ecological importance of pelagic tunicates and their role in marine ecosystem have been receiving increasing attention in the past decades (Nakamura 1998; Cristian and Madin 2004; Alldredge 2005). One of the main characteristics of these organisms is the adaptation to different environmental conditions due to their reproductive cycles and life histories (Alldredge and Madin 1982). They are prey for fishes (Sigler 2001) and they consume bacteria and plankton through suspension feeding (Madin et al. 1997). This way they retain large amounts of particulate material (Sommer et al. 2002),

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even smaller particles. This allows them to alter the carbon flow through the planktonic food web. This makes them also a fundamental link in the microbial food web (Cristian and Madin 2004). With fecal pellets, discarded houses of appendicularians and dead bodies of pelagic tunicates that all sink to deeper waters, they contribute to marine snow and have a strong impact on different biological processes (Andersen 1985; Alldredge 2005). Tunicates are often used as indicators of ocean currents (Chen et al. 1988), also considering their high abundances in certain areas (Nakamura 1998). Pelagic tunicates are also studied with respect to their filtration and clearance rates (Gibson and Paffenhöfer 2000), reproduction and life cycles (Tebeau and Madin 1994), vertical migration and transport of organic matter (Wiebe et al. 1979), and abundance and distribution (Tew and Lo 2005; Deibel Paffenhöffer 2009).

Nuclear Power Plants I and II are located in most populated northern Taiwan. Effects of the discharge of the cooling water from nuclear power plants have drawn great attention in the operation of Nuclear Power Plants I and II in northern Taiwan since 1977 (Wong et al. 1998). There are several studies on the effects of nuclear plants on the different species of planktonic organisms, especially on copepods (Hwang et al. 2004) and on phytoplankton (Lo et al. 2004). No apparent effect of radioactive pollution on marine organisms was found, and no clear and direct relationship between the thermal discharge of nuclear power plants and eutrophication was detected (Huh et al. 2004; Chen et al. 2004). There were negative effects noted directly from the inlet and outlet waters of nuclear power plant cooling water discharges directly on zooplankton abundance and distribution (Hwang et al. 2004). However, these negative effects were limited within 500 meters away from the cooling water discharges. The results of the studies on planktonic communities showed that NE monsoon, ocean currents and topography of the area influence the abundance and distribution of these organisms were much significant than the discharge of cooling waters. However, considering other studies worldwide, the thermal discharge of power plants might have significant effects on plankton and other organisms within small or intertidal areas near the plants and might influence the hydrographic conditions (Sasikumar et al. 1993; Hwang et al. 2004), particularly with the addition of biotic inhibitors into the intake of cooling water (Saravanane et al. 1998; Hwang et al. 2004). The distribution of pelagic tunicates can be influenced by seasonal changes in the water movement and their associated temperature and salinity structure (Kannathasan et al. 2012). In northern Taiwan there are no studies on pelagic tunicates and our research could be a starting point for further research. The choice of stations organized in transects was made in order to understand what kind of effects the discharged cooling waters from the nuclear plants have on species composition, abundance and distribution.

Very little is known about tunicates in the Chinese Seas (Hwang et al. 2004; Kâ and Hwang 2011). Most of the studies are written in Chinese and are concerned with faunistic studies about species composition (Xu et al. 2006). The manuscripts published in English so far are focusing on the abundance and distribution of tunicates (Zhang et al. 2003; Liu et al. 2012; Franco et al. 2014).

The objectives of this study were: 1) to survey the taxonomic species composition of pelagic tunicates in the waters off the two nuclear plants in northern Taiwan; 2) to determine the seasonality of their abundance and distribution patterns at the stations of the chosen transects with different distances from the inlets and outlets of the same nuclear plants; 3) to analyze the correlation between major environmental factors (temperature, salinity and chl-*a*) and the presence of these organisms.

MATERIALS AND METHODS

Zooplankton samples were collected by surface net tows in the waters adjacent to Nuclear Power Plant I and II in Taiwan (Fig. 1). The surveys were conducted with a total of 21 stations; following five transects starting from the inlets and outlets of the two nuclear plants. The study was carried out during one year time, starting in August (summer) and November (autumn) 2014, and March (winter) and May (spring) 2015. The samples were collected on board of the Ocean Research Vessel II, NSC, using a Norpac zooplankton net (180 cm long, 45 cm mouth diameter, 333 μm mesh size). After collection, they were preserved in 5% buffered formalin. Data on temperature, conductivity and chlorophyll-*a* were obtained by a CTD (Sea-Bird SBE 911). Later in the lab, identification and counting of the species for each sample were performed using a stereomicroscope (Olympus, SZX16, Tokyo, Japan). The abundance is expressed as ind./m^3.

All data mapping concerned with abundance and distribution was made using the software SURFER 8.0. In order to determine the relationship between the abundance and distribution of pelagic tunicates and environmental factors, and to understand the effects of discharged cooling waters on the organisms, a stepwise regression model was carried out with sea surface temperature, surface salinity and chl-*a* concentration as independent variables and the total abundance of the two main classes of pelagic tunicates (Larvacea and Thaliacea) as independent variables during four seasons (for the procedure see Tew and Lo 2005). The peculiarity of the model used here was that we firstly checked the relationship between the variables following transects from inshore to offshore. Secondly, we tried to analyze the same relationship taking into consideration the stations going from the NPPI, located in the northern part of the area, towards the NPPII, located at lower latitudes. The computer program Eviews 6.0 software was used for all the calculations.

RESULTS

Environmental conditions

The variations of mean surface temperature and salinity during the four seasons in the studied area are shown in figure 2. The changes of salinity are limited, ranging from 32.9 to 34.4. As for the surface temperature, there is a significant change during the four seasons. In summer, the mean temperature for the entire area was 28.2°C, while in winter it decreased to 18.4°C. The values for the autumn and spring seasons

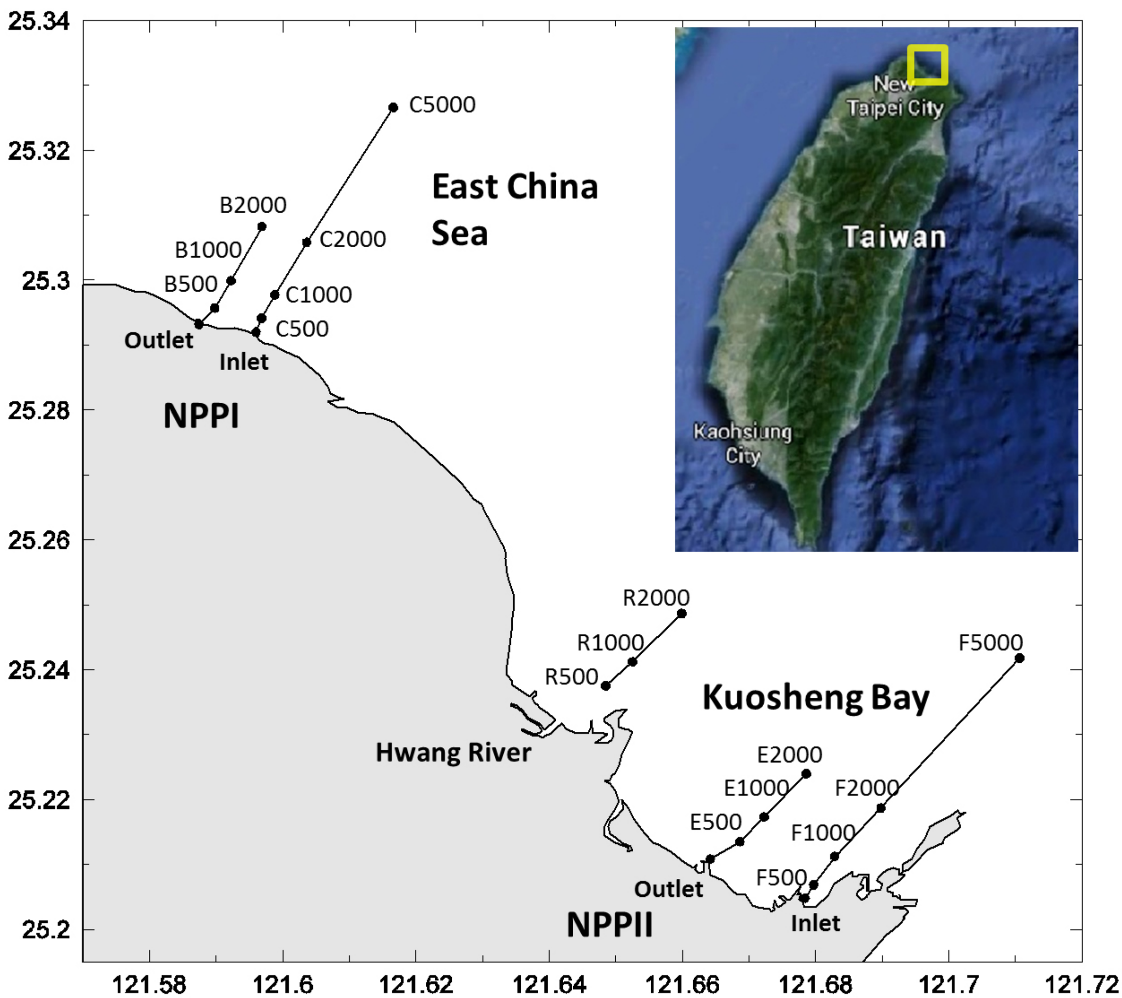

Fig. 1. Location of sampling stations in the waters off NPPI and NPPII of Northern Taiwan. The letter and number of each station represent transect and distance (m) from land.

were 22.6°C and 23.7°C, respectively. What was interesting to observe was that water surface temperature did not vary significantly over the area. In fact, surveying all the stations within one season, we could find a difference of temperature values of maximum 1°C. The same happened for salinity values. The only places where surface temperature and salinity were significantly different were the ones measured in the two outlets of the nuclear plants. The temperature gradients from outlet to offshore were not significantly different. In any case, in these areas we could not find any tunicates.

The NPPI and NPPII show two different hydrography systems. The waters out the NPPI can be considered as an open sea system (Hu 2004). Instead, the NPPII was built inside the Kuosheng Bay.

Figure 3 shows the changes in chl-*a* concentration during the four seasons throughout the area, covering all 21 stations. In summer, the highest values were measured in the waters off the river mouth and in Kuosheng Bay, reaching up to 2 μg/l. In autumn, the high values (max. 0.6 μg/l) were also detected in the waters off the river month. In winter, the concentrations were extremely low, and maximum values (0.4 μg/l) were found within the bay. Finally in spring, the Kuosheng Bay showed the most significant concentrations of chl-*a* (max 0.8 μg/l).

Taxonomic composition, spatial distribution and abundance

A total of nine species were identified: *Oikopleura dioica*, *O. caphocera*, *O. rufuscens*, *O. longicauda*, *Fritillaria aberrans* belonging to the class Appendicularia and *Dolioletta denticulatum*, *Thalia democratica orientalis*, *D. gegenbauri* and *Thetys vagina* belonging to the Thaliacea. When the Appendiculariidae could not be identify to the species level, we referred to them as *Oikopleura* sp. Table 1 shows a list of the species encountered during the survey and their abundances. The entire area was categorized into three different zones (NPPI, NPPII and Hwang River). NPPI and NPPII include two different transects. For every species, the table shows the relative mean abundance in percentage (%) at every zone or the four seasons. The last row shows the total mean abundances for every zone expressed in ind./m^3. The family Appendiculariidae, belonging to the class Larvacea, was the most abundant throughout the area, with the highest abundance values measured during summer. In this season, *O. dioica* and *O. longicauda* were the dominant species. Several larvaceans could not be identified at species level and high percentages of *Oikopleura* sp. were present. During the rest of the year, the concentrations of pelagic tunicates were significantly low. In autumn, the thaliaceans *D. gegenbauri* belonging to the family Doliolidae and *Thalia democratica orientalis* belonging to the family Salpidae were the dominant species. In winter we measured the lowest concentrations and larvaceans were relatively most abundant. Finally, during spring, we found a distribution depending on the characteristics of the area. In fact, at transacts of NPPI, *Doliolium denticulatum* had the highest concentrations; at Hwang river, it was *O. dioica* which showed dominance; at NPPII, the doliolid *Dolioletta gegenbauri* was the most abundant.

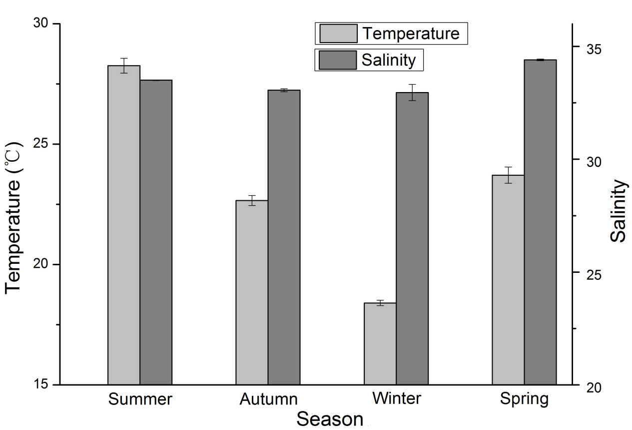

Fig. 2. Graph showing the changes in temperature (°C) and salinity during four seasons.

Figures 4 and 5 show the distribution and abundance of pelagic tunicates during the four seasons. We grouped all the species into the two main classes they belong to: Larvacea and Thaliacea. Larvacean concentrations were abundant during summer, especially at stations along transacts of Hwang River and NPPII. Thaliaceans were more present during autumn, even though their concentrations were significantly low during the entire period. Pelagic tunicates tended to be more abundant in the Kuosheng Bay and in the waters off the river mouth.

Pelagic tunicates and environmental variables

Figure 6 shows the total mean abundances of pelagic tunicates, total average temperature and chl-*a* values in the study area. The highest concentrations of these organisms were measured during summer (mean value: 561 ind./m³), when the values for surface temperature and chl-*a* were detected (mean surface temperature: 28.2°C; mean chl-*a*: 0.96 μg/L). We found for spring and autumn seasons similar values for environmental conditions as well as for organism abundance levels. The mean surface temperatures measured for the two seasons was 22.6°C for autumn and 23.7°C for spring. The mean abundances were 4.1 ind./m³ for autumn and 2.2 ind./m³ for the spring season. In winter, the low temperatures (mean value:18.4°C) and chl-*a* (mean value: 0.25 μg/L) were associated with extremely low values of organism abundances (mean value: 0.3 ind./m³).

A stepwise regression was used to determine which environmental factors were the most significant ones to influence the abundance and distribution of tunicates (grouped in the two

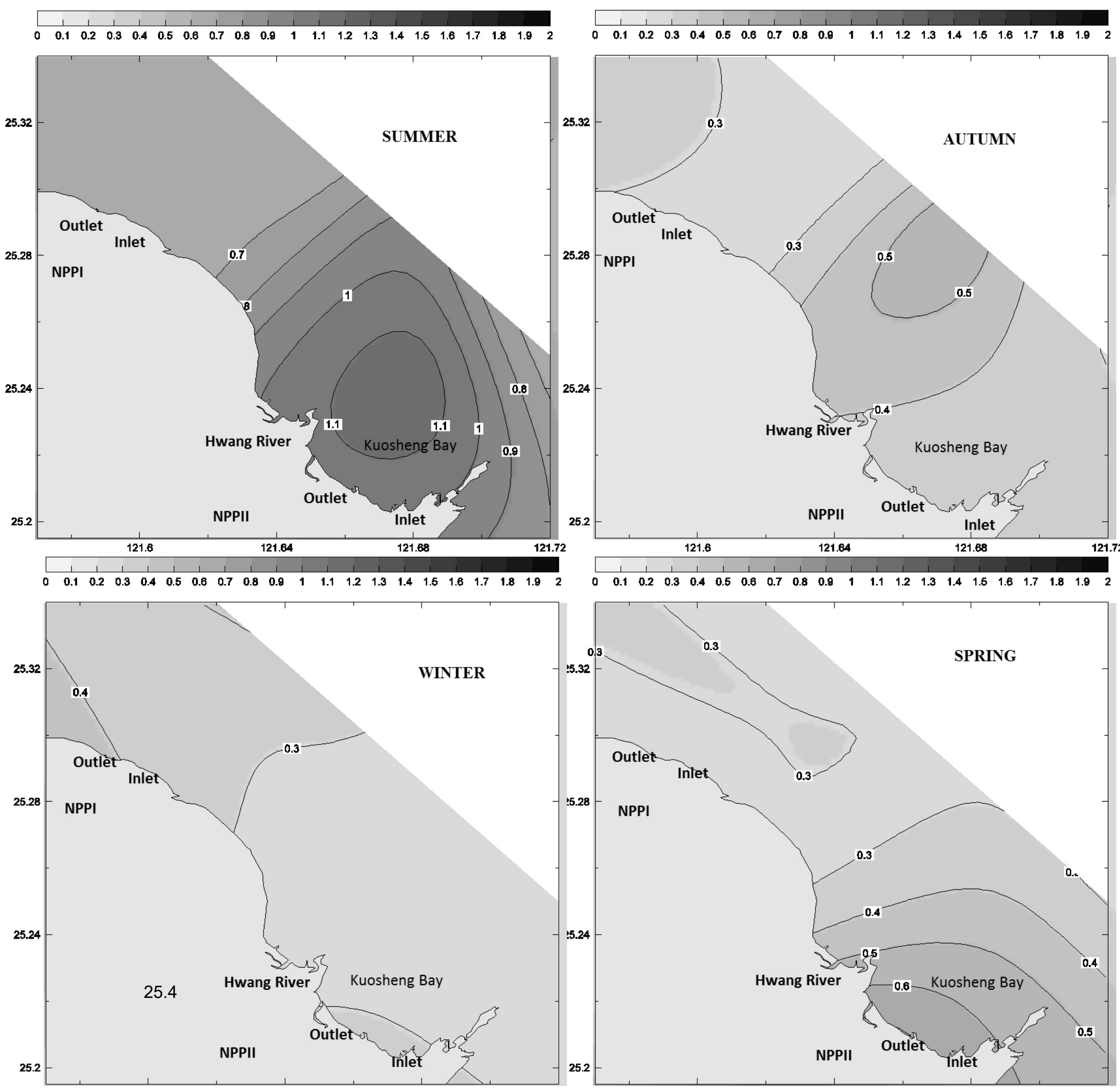

Fig. 3. Map showing the concentration of chl-*a* in the study area during four seasons.

taxonomic classes Larvacea and Thaliacea) in the area off the nuclear plants. The surface temperatures, surface salinities and chl-*a* concentrations were selected as independent variables, whereas the abundance of tunicates was selected as the dependent variable. The correlations were expressed by the function $y = \beta x + c$.

No relationship was found between the abundance of tunicates and surface temperature, salinity and chl-*a* comparing the values of all four seasons. Neither was a relationship found between tunicate abundance and surface temperature and salinity in the area within one season, likely due to slight changes of the two environmental parameters. The abundances of larvaceans and thaliaceans were significantly influenced by the concentration of chl-*a*, among the four seasons as well as within all stations for each season. This correlation regarding the data for each

Table 1. Distribution of the different species during the four seasons at the different transects

	Relative abundance (%)					
Zooplankton	SUMMER			AUTUMN		
Class: Larvacea	NPPI	NPPII	Hwang river	NPPI	NPPII	Hwang river
Family: Appendiculariidae						
O. dioica	23.4	36	17.2	4.2	29.5	5.8
O. longicauda	29.4	20.6	12.6	-	-	-
O. rufuscens	9	23	1.5	-	-	-
O. caphocera	0.7	3.3	3.1	-	-	-
Oikopleura sp.	36.3	11.3	62.6	4.2	7.5	1
Family: Fritillariidae						
F. aberrans	0.6	1.3	1.1	-	-	-
Class: Thaliacea						
Family: Doliolidae						
D. denticulatum	-	0.1	-	0.7	0.6	0.7
D. gegenbauri	0.4	1.9	1.2	41.6	37.3	21.3
Family: Salpidae						
T. democratica orientalis	0.2	2.5	0.7	46.5	24.8	70.5
T. vagina	-	-	-	2.8	0.3	0.7
Total amount (ind./m^3)	**25.74**	**653.06**	**1146.33**	**1.42**	**3.18**	**10.28**

	Relative abundance (%)					
Zooplankton	WINTER			SPRING		
Class: Larvacea	NPPI	NPPII	Hwang river	NPPI	NPPII	Hwang river
Family: Appendiculariidae						
O. dioica	71.7	41.9	65.2	32.5	66.7	24.4
O. longicauda	5	24.2	-	11	8.4	6
O. rufuscens	-	-	-	-	-	-
O. caphocera	-	-	-	-	-	-
Oikopleura sp.	23.1	32.2	26.9	6.1	2.6	7.1
Family: Fritillariidae						
F. aberrans	0.1	0.6	7.7	-	-	-
Class: Thaliacea						
Family: Doliolidae						
D. denticulatum	-	-	-	40.5	12	12
D. gegenbauri	0.1	1.1	0.2	6.1	7.4	50.2
Family: Salpidae						
T. democratica orientalis	-	-	-	3.7	1.9	0.2
T. vagina	-	-	-	0.1	1	0.1
Total amount (ind./m^3)	**0.39**	**0.62**	**0.26**	**1.63**	**3.09**	**2.34**

single season was calculated considering the five transects from inshore to offshore waters, but also the same area going from the north-positioned NPPI to the south-positioned NPPII. In the first case, we could not find any correlation. In the second case, the correlation was significant during summer and spring. In summer, the correlation function was expressed by the equation y = 2116.4x-1328.6 with the R^2 value equal to 0.8903. In spring, the correlation function was expressed by the equation y = 5.782x-1.0366 with the R^2 value equal to 0.9247. Considering the results of our statistical model, we show the spatial distribution and abundances of pelagic tunicates as well as the chl-*a* concentrations over the area in summer and spring in figures 7 and 8, in order to have a better view of their relationship.

DISCUSSION

The present study was focusing on the taxonomic composition, spatial distribution and abundance of appendicularians, salps and doliolids

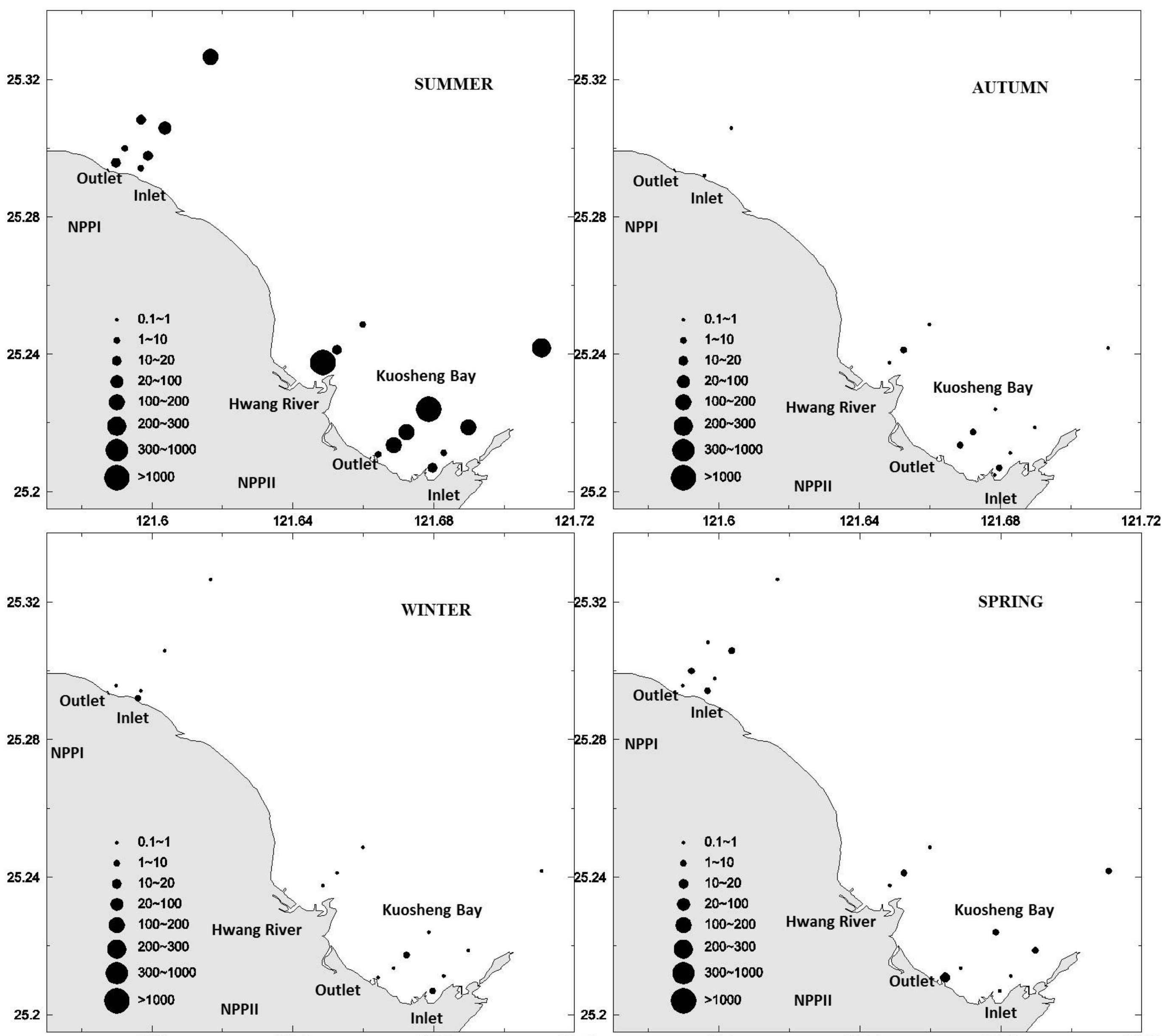

Fig. 4. Distribution and abundance of Larvacea during four seasons.

in the waters off the nuclear plants in northern Taiwan and on their occurrence, characterized by strong seasonality, and environmental factors. Figure 6 shows an overview of the obtained results. There is a strong seasonality in the occurrence of pelagic tunicates in the area. In fact, in summer, the abundances of these organisms were high in most of the sampling stations. The values measured in summer (average value: 561 ind./m^3) are of a much superior magnitude compared to the other seasons (autumn: 4.1 ind./m^3; winter: 0.3 ind./m^3; spring: 2.2 ind./m^3). A high variability in our data prevented the use of statistical methods that could analyze the relationship between temperature and chl-*a* changes and the abundance of the organisms throughout the four seasons. But looking at figure 6, it becomes clear that the pattern of occurrence of gelatinous zooplankton follows the changes of the same environmental parameters. We can see a difference between the two classes Larvacea and Thaliacea. The Larvacea, with the family Oikopleuridae shows highest concentrations. Representatives of the family Oikopleuridae are

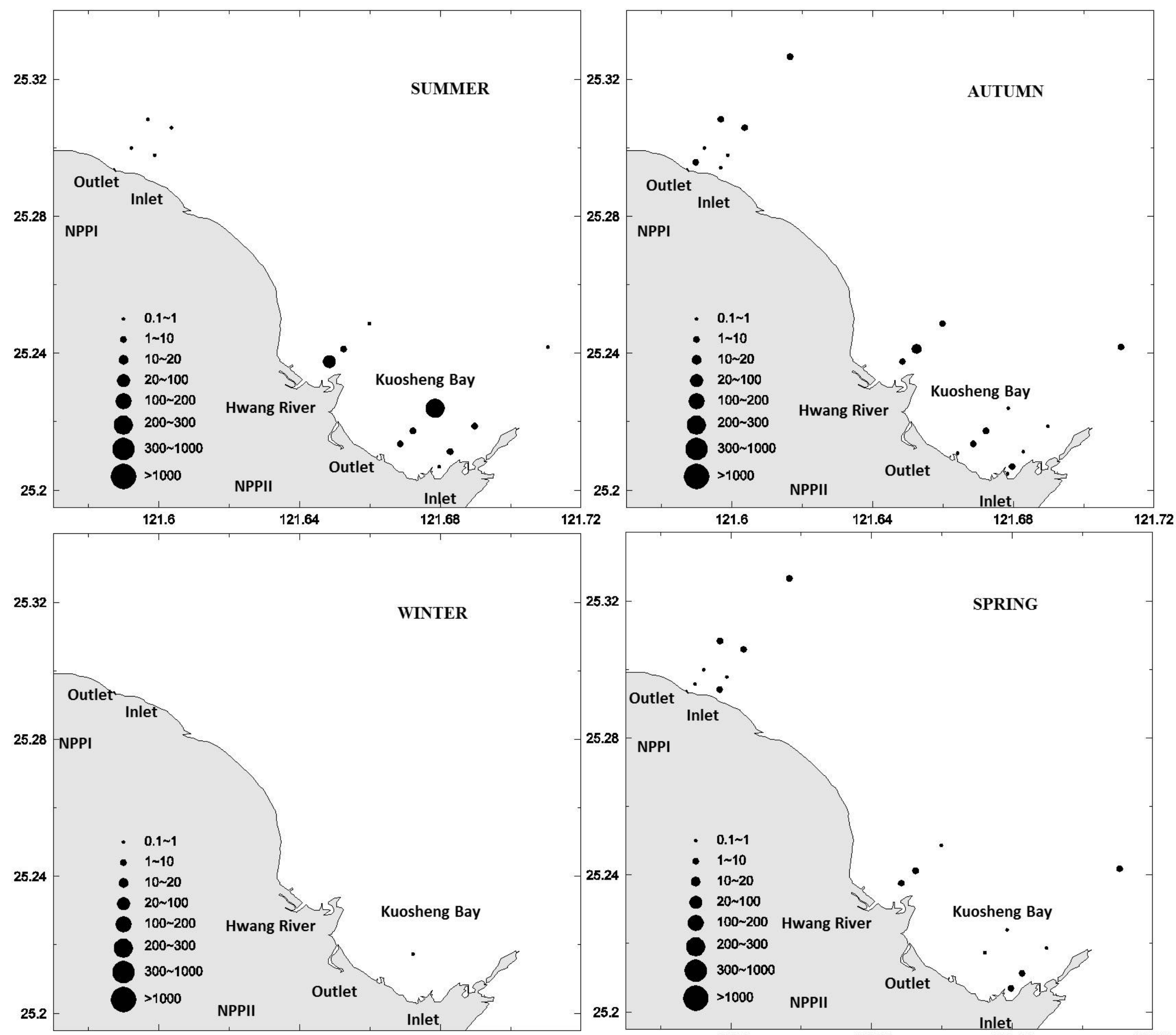

Fig. 5. Distribution and abundance of Thaliacea in the study area in NE Taiwan during four seasons.

eurythermal and euryhaline (Alldredge 1976). The reproduction of appendicularians is proven to increase with a rise in temperature (Paffenhöfer 1976) and the two most abundant species are known to prefer surface water for spawning (Alldredge 1976). Nakamura showed that both Larvacea and Thaliacea can show high patches of abundances in different areas, thanks to the filter feeding capacity and depending on the availability of food. Despite this, the concentrations of thaliaceans were not significantly different during summer even although the identified species are known as warm-water species. The larvaceans can replace their clogged feeding apparatus, called 'house', when food particles are too dense (Tiselius et al. 2003). Thaliaceans are also filter-feeders but their apparatus could be clogged and damaged because of abundant levels of particulate material (Zeldis et al. 1995). We hypothesize that high patches of larvaceans influenced the distribution of thaliaceans during the warm season as competitors for food. At the same time we found both availability of food and surface temperature affect the zooplankton abundances in the area

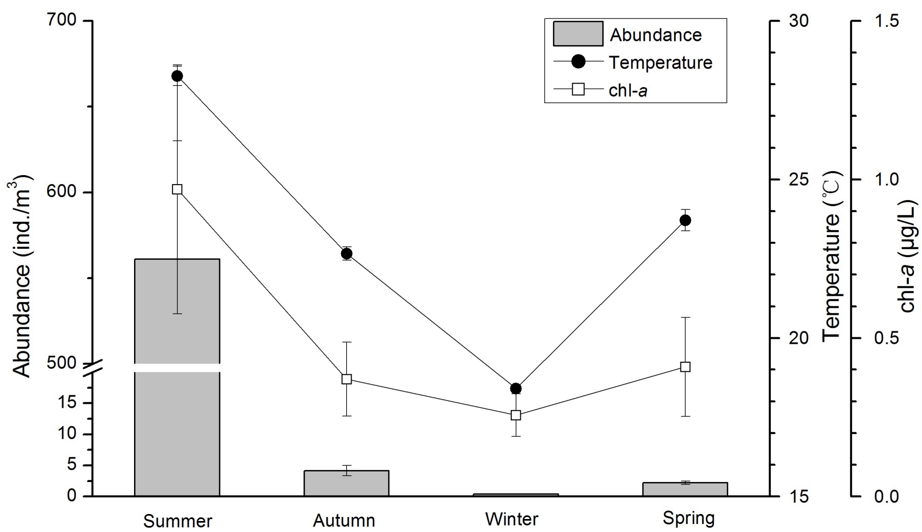

Fig. 6. Graph showing the total abundance of pelagic tunicates, the total average temperature and chl-*a* in the study area.

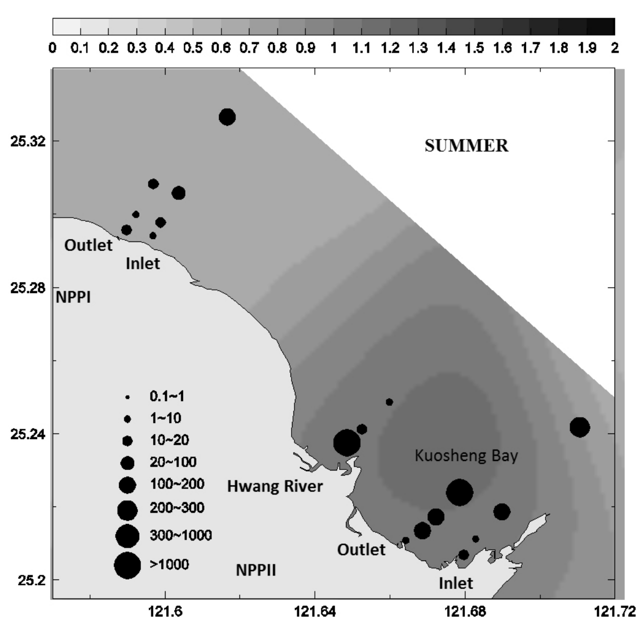

Fig. 7. Distribution of pelagic tunicates and Chl-*a* during summer

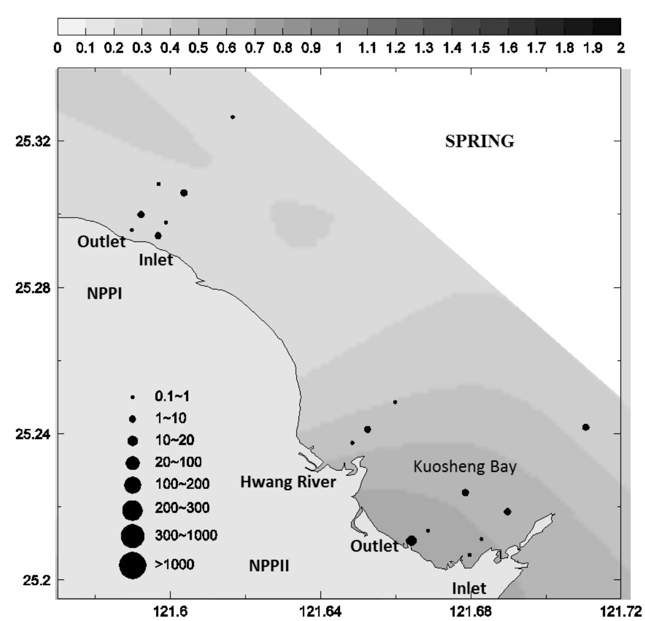

Fig. 8. Distribution of pelagic tunicates and Chl-*a* during spring

throughout the study period. Another reason for thaliaceans to have a low concentration during the four seasons, might be due to their ecology and reproductive behavior. Doliolids (belonging to the class Thaliacea) do not show clear diel vertical migration (Gibson and Paffenhöfer 2000), but they can live in different water depths. The doliolids *D. denticulatum* and *D. gegenbauri* show alteration between sexual and asexual reproduction. Every life stage tends to stay at different water depths (Tew and Lo 2005). As for salps, they show vertical migration, which may allow the organisms to approach the surface in order to feed and then migrate to deeper waters which are more suitable for their life conditions.

Our survey had the initial objective of measuring and understanding the influence of the discharged thermal cooling waters from the nuclear power plants and its effect on tunicates in surface waters. This hypothesis could be discarded. In fact, the variations of surface temperature and salinity in each season throughout the area were negligible, with maximal ranges of 1°C temperature and constantly low salinity values. A survey from deeper waters could show different results, especially considering the distribution of these organisms. In fact, doliolids and salps can migrate to deeper environments during their life cycles. For this it would be necessary to conduct a different approach in the area, perhaps using vertical tows sampling the entire water column and considering the changes in temperature and salinity throughout the area.

The last part of our study was concerned with a correlation of season and the abundance of pelagic tunicates as well as environmental factors. As mentioned, surface temperature and salinity do not vary much within each season in the overall area. Chl-*a* does vary in the studied area. Considering the topography and hydrography of the area, the waters off the NPPI can be considered as an open sea system (Hu 2004). In contrast, the NPPII was built inside the Kuosheng Bay. The discharge of the Hwang river into the sea is also an important factor to consider. The highest abundances of organisms were measured in the waters inside the Kuosheng Bay and river estuary. We found a significant correlation between the latter and availability of food in the area in summer and spring (Figs. 7-8), moving our attention from the north (NPPI) towards the south (NPPII). We found no correlation between organism abundances and chl-*a* considering the values immediately from the coast towards the open ocean. Our results are in concordance with several related studies. Deibel and Paffenhöffer (2009) measured extremely high abundances of tunicates in upwelling waters which were rich in nutrients and phytoplankton. Nakamura (1998) and Cristian and Madin (2004) stated that Thaliaceans tend to congregate in the areas of high abundances of nano- and ultra-plankton. Franco et al. (2014) found that high abundances of Larvaceans in the North Yellow Sea occurred in areas with high chl-*a* concentrations and affirmed that the latter were not limiting factors for this taxon which is able to substitute its feeding structure (house).

CONCLUSIONS

Our results showed that pelagic tunicates occurred in the research area at high abundances during the summer season and the main environmental factor influencing their presence is the availability of food during each season throughout the area, and both surface temperature and chl-*a* when we consider all four seasons. It is important to analyze the community structure and its connection to the oceanic environment: firstly, in order to describe the biodiversity of the same area; secondly, and most important, to be able to understand the role of zooplankton in their ecological niches, the links of zooplankton with other oceanic organisms and their significance in the oceanic food web. We could refute the initial hypothesis that discharged cooling waters from nuclear power plants affected the distribution of pelagic tunicates in surface waters. It is necessary to conduct a different research on the area, perhaps using a vertical tow in the entire water column and considering the changes in temperature and salinity throughout the area. We suggest to use the warm water species *D. denticulatum* as a reference for current circulations as this species was recorded in different places in the China Seas.

There are some limitations to our findings. Our research included samples covering four seasons within a two-year period (2006-2007). As our survey is the first one in the area, it is not possible to compare our results with those of others. The collection of organisms was carried out in surface waters and not through a vertical tow in the water column. As mentioned, there are tunicates which migrate or reproduce in deeper waters. Furthermore, the mesh size of the plankton net (333 μm) was not fine enough to collect smallsized organisms, including new-born and juveniles. Nevertheless, are we expecting our study to be a starting point for further research. It would for instance be interesting to measure the effects of predation by fish larvae or other organisms on tunicates. This could elucidate the efficiency of their grazing and filter-feeding, to analyze their behavior in the laboratory or *in situ*, and to consider their reactions to food limitation or changes in physical environmental conditions. Moreover, future work on abundance and distribution shifts caused by climate changes in addition to thermal discharge effects at different seasons could be helpful to better understand expected changes of the pelagic tunicate assemblage structure. These and other topics should be taken into consideration in order to have a better understanding of the entire pelagic ecosystem in the waters of northern Taiwan.

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