

## EFFECTS OF VARIATIONS OF ENVIRONMENTAL FACTORS AND ALGAL BLOOM ON FISH LARVAE IN SOUTHERN CENTRAL VIETNAM

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**ABSTRACT:** A multivariate analysis was conducted to assess the impact of variations of environmental factors, such as temperature, salinity, dissolved oxygen (DO), total suspended solid (TSS), nitrite (NO<sub>2</sub>-N), nitrate (NO<sub>3</sub>-N), ammonium (NH<sub>3,4</sub>-N), phosphate (PO<sub>4</sub>-P), and silicate (SiO<sub>3</sub>-Si) concentrations, chlorophyll (chlor) and algal bloom on natural fish larvae in Binh Thuan waters, Vietnam. Temporal and spatial variations of environmental factors were correlated with the abundance and diversity of fish larvae. The abundance and diversity of fish larvae decreased at the sites of the algal blooms. Five environmental factors: temperature, salinity, nitrite, phosphate and silicates showed a statistically significant impact on the fish larvae, but other factors showed weaker and statistically not significant effects. In the months of algal blooms, phosphate, silicate, nitrite, nitrate, ammonium and salinity influenced blooms substantially, while the effects of temperature were mainly confined to the months without blooms of algae. Algal blooms were lethal to fish larvae and juveniles leading to a decline in their abundance and diversity. This will affect and restrict the process of recruitment of fish stocks in the region.

*Keywords:* Fish larvae, algal bloom, environmental factors, South Central Coast, Vietnam.

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### INTRODUCTION

Algal blooms have occurred in Binh Thuan waters previously. A serious harmful algal bloom (HAB) caused by a Haptophyte, *Phaeocystis* cf. *globosa*, occurred over 30 km of northern coastal waters of Binh Thuan Province in July 2002, and about 90% of animal and plant species in tidal reefs of Phan Ri Bay were destroyed by this bloom, causing a economic loss of over VND 10 billion (ca. \$US 650,000). Laboratory studies elucidated the toxicity of this algal species and its impact on embryonic development of and lethality for fish larvae. The mechanisms leading to fish death was suggested by Bruslé (1995), Chen (2001), Gosseline (1989), and Salierno (2006) and the influence of *P. globosa* on the recruitment ability of fish populations was noted previously (Robineau, 1991).

Binh Thuan waters are known as highly productive fishing grounds with fish

communities having a varieties of species with high abundances. It is one of the four provinces with the largest seafood production in the country (Le et al., 2001). In 2007, fishing production was 90,400 tons (General Statistics Office, 2010). In Binh Thuan waters the relationship between fish eggs and larvae have been investigated in programs such as Project: Thuan Hai - Minh Hai (1978-1980), KC.03-05 (1992-1995) and Vietnam - German protocol (2003-2005). Binh Thuan waters are spawning grounds, with high densities of eggs (more than 1000 eggs/100 m<sup>3</sup> (Nguyen, 1997; Vo et al., 2004). In most previous surveys, fish eggs and larvae have been assessed for the potential for recruitment. However, the impacts of harmful algal blooms (HABs) on the recruitment ability of fish populations have not been focused on.

Surveys during 2007-2008 were initially conducted to investigate the decline in the diversity and abundance of larvae. However, at that time, substantial algal blooms had

happened. The purpose of this paper is to address the impact of algal blooms on larval and juvenile fish through changes of their diversity and abundance in relation to the appearance of algal blooms. This study will provide better understanding about the extraordinary impact of toxic algal blooms in the sea.

## MATERIALS AND METHODS

### *Sampling and analyzing samples*

In the framework of the project KC09.03/06-10, sampling stations were along transects from inshore to offshore belong to areas of Vinh Hao, Phan Ri, Hong Phong, Phan Thiet and Ham Tan (fig. 1).

The ichthyoplankton were sampled during six field-trips in 2007 (from May to October) and four times in 2008 (from April to July). At each station, samples were collected once per month. In addition, only in July 2008, sampling was performed more than once at the station DS5. The larval fish samples in surface waters were collected using a rectangular net (dimensions: 90 × 56 cm; area: 0.5 m<sup>2</sup>)

equipped with a flow meter on the mouth. The net was towed for 10-15 minutes at 2-3 knots using a fishing boat. All samples were preserved in 5% formalin immediately in the field and then, they were sorted to separate fish larvae from plankton samples. The taxa of fish larvae were identified under a stereomicroscope SZ7, Olympus (Japan) referring to the literatures such as Okiyama (1988), Leis and Rennis (1983), Leis and Trnski (1989), Leis and Carson-Ewart (2004), and Neira et al. (1998). The average density of fish larvae was calculated as the number of individuals per 100 m<sup>3</sup>.

Hydrographic factors such as temperature (T), salinity (S) and chlorophyll-a (chl<sub>a</sub>) were measured using a CTD profiler (SeaBird 19<sup>+</sup>, USA). Dissolved oxygen (DO), total suspended solid (TSS), nitrite (NO<sub>2</sub>-N), nitrate (NO<sub>3</sub>-N), ammonium (NH<sub>3,4</sub>-N), total phosphate (PO<sub>4</sub>), and silicate (SiO<sub>3</sub>) were analyzed in the laboratories of the Institute of Oceanography according to the Standard Methods For Examination of Water and Waste Water. 21<sup>st</sup> Edition (2005).

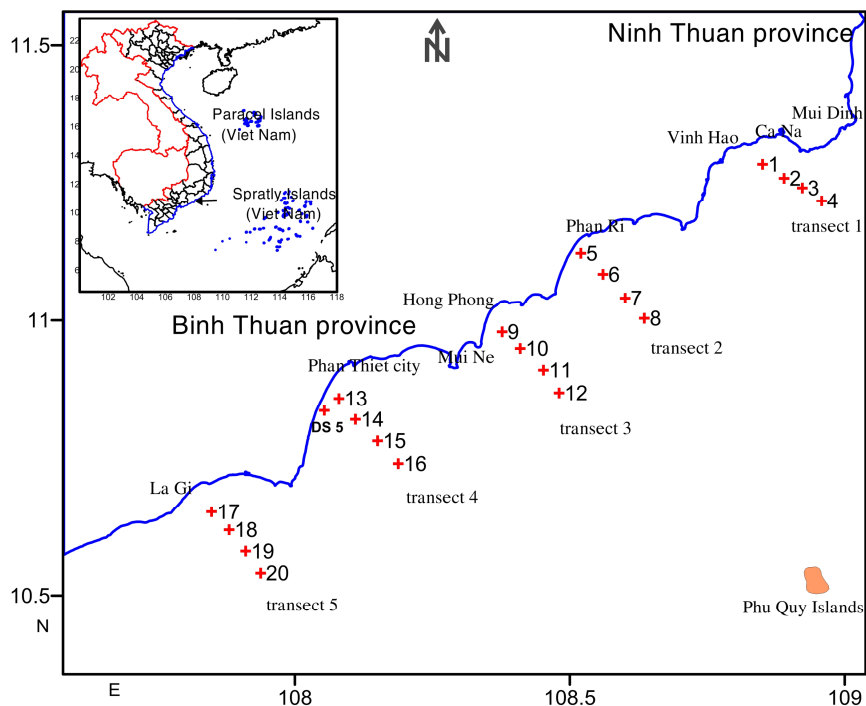


Figure 1. The map with sites collected fish larvae samples

### Statistical analysis

The influence of environmental factors on the abundance, diversity and temporal changes of fish larvae in relation to the algal bloom characteristics was analyzed using the Canonical Correspondence Analysis (CCA) of the 10 environmental factors mentioned above (Jongman et al., 1995; ter Braak, 1986; ter Braak and Verdonschot, 1995). The non-biological and biological parameters used for multivariate analyses include temperature, salinity, dissolved oxygen (DO), total suspended solid (TSS), nitrite (NO<sub>2</sub>-N), nitrate (NO<sub>3</sub>-N), ammonium (NH<sub>3,4</sub>-N), total phosphate (PO<sub>4</sub>), silicate (SiO<sub>3</sub>), chlorophyll-a (chlor) and the fish larvae diversity and abundance, which included 80 taxa (family, genera and species).

The calculations were performed using the

Canoco 4.0 software package (Jongman et al., 1995; Lepš and Šmilauer, 1999; Lepš and Šmilauer, 2003).

## RESULTS AND DISCUSSION

### Environmental parameters

Concentrations of nutrients such as nitrate and phosphate were highest in August 2008 with values of 43.78 and 23.20 µg/l respectively, nitrite was highest (4.17 µg/l) in June 2008, ammonia was highest (28.10 µg/l) in September 2007, and silicate was highest (443.75 µg/l) in April 2007. The range of variation of the temperature and salinity of the surface waters were 4.25°C and 1.56 (‰), respectively. Chlorophyll-a was highest in August 2007, while dissolved oxygen (DO) was lowest in September 2007 (table 1).

Table 1. The mean (± SD) of environmental parameters on surface waters

Parameters	2007						2008			
	May	June	July	Aug.	Sept.	Oct.	April	May	June	July
Temperature (°C)	28.84 ± 0.84	29.59 ± 0.35	29.36 ± 0.75	26.41 ± 1.06	27.67 ± 0.46	28.60 ± 0.24	29.31 ± 0.33	28.15 ± 0.46	25.34 ± 0.24	26.44 ± 1.38
Salinity (‰)	33.52 ± 0.27	32.36 ± 0.57	32.30 ± 0.38	33.24 ± 0.59	33.36 ± 0.21	32.39 ± 0.42	33.30 ± 0.38	33.25 ± 0.27	33.86 ± 0.05	33.57 ± 0.35
Dissolved oxygen (mgO <sub>2</sub> /l)	6.17 ± 0.10	6.19 ± 0.23	6.23 ± 0.30	6.96 ± 0.99	5.80 ± 0.60	6.10 ± 0.09	6.30 ± 0.08	6.56 ± 0.10	7.10 ± 0.17	6.11 ± 0.24
Chl-a (µg/l)	0.55 ± 0.36	0.53 ± 0.44	0.31 ± 0.13	3.46 ± 1.96	1.73 ± 0.99	0.82 ± 0.48	0.44 ± 0.35	0.36 ± 0.29	1.27 ± 0.24	2.47 ± 2.10
TSS (mg/l)	3.12 ± 1.57	3.51 ± 1.69	2.75 ± 1.99	2.59 ± 1.19	11.70 ± 4.60	1.97 ± 1.05	3.14 ± 5.99	2.21 ± 2.91	3.24 ± 1.12	2.20 ± 1.01
NO <sub>3</sub> -N (µg/l)	29.26 ± 8.41	38.38 ± 5.78	36.64 ± 3.04	34.08 ± 2.99	35.55 ± 3.45	33.5 ± 1.09	32.87 ± 2.12	33.57 ± 1.12	34.46 ± 2.38	43.78 ± 10.92
NO <sub>2</sub> -N (µg/l)	2.88 ± 6.12	1.61 ± 0.58	0.986 ± 0.54	1.478 ± 2.42	0.725 ± 1.10	1.521 ± 0.72	0.912 ± 0.63	0.284 ± 0.66	4.17 ± 1.10	1.210 ± 2.451
NH <sub>3,4</sub> -N (µg/l)	5.0 ± 9.14	5.42 ± 8.20	0.26 ± 0.79	6.89 ± 12.5	28.10 ± 11.70	4.63 ± 6.52	8.12 ± 7.71	1.07 ± 1.37	3.06 ± 3.69	3.57 ± 5.86
PO <sub>4</sub> -P (µg/l)	11.57 ± 2.08	6.105 ± 2.07	8.266 ± 2.72	15.33 ± 5.16	13.3 ± 3.23	13.42 ± 1.97	3.081 ± 0.96	9.642 ± 1.83	5.993 ± 2.49	23.20 ± 3.06
SiO <sub>3</sub> -Si (µg/l)	198.1 ± 46.75	253.4 ± 120.3	325.06 ± 118.5	345.57 ± 114.1	342.2 ± 91.43	312.42 ± 88.93	±	227.84 ± 89.44	233.46 ± 65.89	286.78 ± 65.00

### The composition and density of fish larvae

The population of fish larvae found was relatively diverse, including a total of 80 taxa belonging to 70 families. The dominant taxa are tiger fish (Theraponidae); followed by gobies (Gobiidae); Smelt-whitings (Sillagidae); and

Dragonets (Callionymidae). Most of them had a high proportion of larvae in all months sampled. Twenty families accounted for a large proportion of the total abundance (77.7%) among the 70 families of fish identified, and 50 other families accounted for 22.4% (fig. 2).

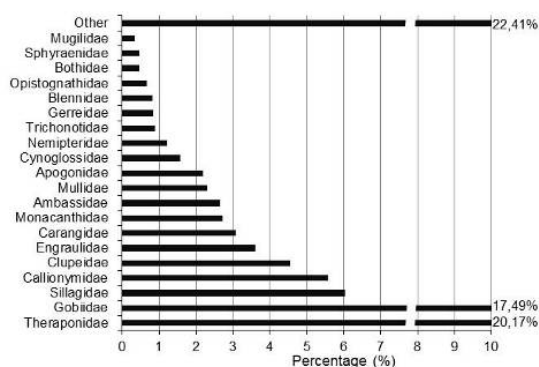


Figure 2. The percentage of total abundance of families of fish larvae

The average density of fish larvae was lowest in July and August 2007 and July 2008 when was the time of algal blooming. It was also reduced in May 2008 but peaked in October, 2007 (fig. 3). These changes were reflected to the diversity of the taxa of fish larvae, which was also reduced at the time of algal blooming. In July and August, 2007 and also July, 2008, fish larvae were absent in most of the stations.

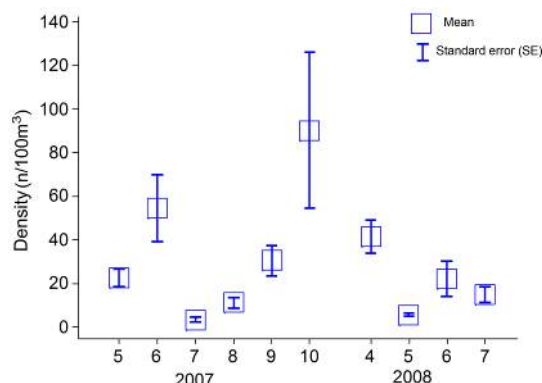


Figure 3. The average density of fish larvae for month in 2007 & 2008

**Correlations between environmental factors and fish larvae**

Our analysis of 10 factors of surface waters show only five environmental factors - temperature, salinity, nitrite (NO<sub>2</sub>-N), phosphate (PO<sub>4</sub>-P) and silicate (SiO<sub>3</sub>-Si) - have a significant (p<0.05) impact on community composition. The values of the parameters of the CCA analysis are given in table 1.

Table 1. Results of CCA analysis of 10 environmental factors (see below)

Axes	1	2	3	4	Total inertia
Eigenvalues	0.236	0.140	0.103	0.086	9.787
Species-environment correlations	0.770	0.730	0.610	0.609	
Cumulative percentage variance of species data	22.4	39.8	48.9	56.8	
Cumulative percentage variance of species-environment relation	26.3	42.0	53.4	63.0	
Sum of all eigenvalues					9.787
Sum of all canonical eigenvalues					0.896

Environmental factors examined: temperature (T), salinity (S), dissolved oxygen (DO), total suspended solids (TSS), total nitrite (NO<sub>2</sub>-N), total nitrate (NO<sub>3</sub>-N), total ammonia (NH<sub>3,4</sub>-N), total phosphate (PO<sub>4</sub>-P), total silicate (SiO<sub>3</sub>-Si) and chlorophyll (Chlro).

The first two CCA axes explained 42% of the cumulative variance of the species-environment relation. The first CCA axis (Eigenvalue = 0.236) alone figured out 26% of the total variance, demonstrating a high species-environment correlation (0.770) (table 1). The

second axis represented 15.7% of the variance, while the third and fourth axes additionally explained > 9% of the variance each (table 1).

The results of CCA are displayed in an ordination diagram, where the axis 1 and 2 indicate months, together with the vectors of sampling sites and environmental factors. The high eigenvalue with 9.787 explained the variance of the station group where the sampling was performed each month with environmental gradients. The environmental variables are displayed by vectors (arrows) of

which lengths scale with the importance of the factors explaining the variation of species composition. The temperature, salinity and phosphate ( $\text{PO}_4\text{-P}$ ) variables correlated strongly with the first CCA axis, and nitrite ( $\text{NO}_2\text{-N}$ ) and silicate ( $\text{SiO}_3\text{-Si}$ ) were mostly correlated with the second CCA axis. The sites that were far away from each other had substantially different species composition. Stations sampled during a single survey were more correlated. It can be seen from figure 4 that stations in May 2007, April 2008 and May 2008 are in the same group. June 2007, July 2007 and Oct. 2007 are within an equivalent group. The difference over time in the composition of fish larvae in the samples collected within two months of the year also reflects the impact of changes in various environmental factors. At the same time, the species composition of fish larvae in June 2007 is contrasted to June 2008; in 2007 the *Phaeocystis globosa* bloom grew and expanded more slowly in August & September, compared with algal blooms in July 2008.

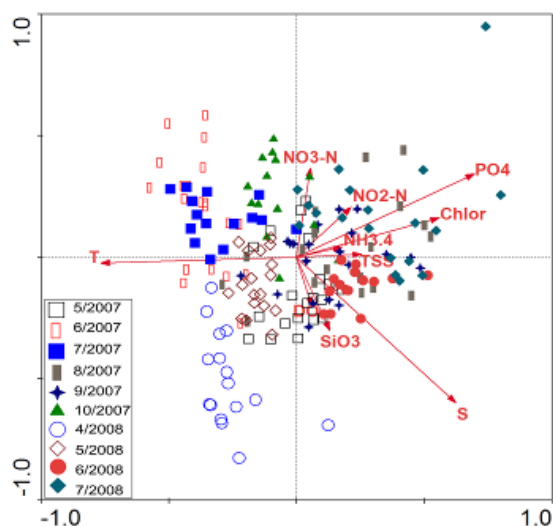


Figure 4. The sample-environmental variables biplot with month classification

The CCA analysis also showed no clear separation of larval composition between the survey transects (fig. 5). Location of samples collected in distinctive areas mixed into each other ordinations and does not suggest a coherent group (fig. 5). This suggests that algal

blooms are not spread throughout the region, but only occur in a few localized areas at different times. This is consistent with satellite imagery of pigment concentrations of the area, which show substantial spatial and temporal variability.

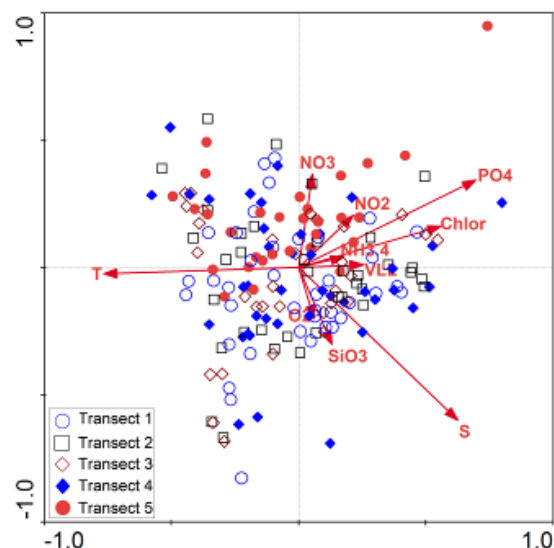


Figure 5. The sample-environmental variables biplot with transect classification

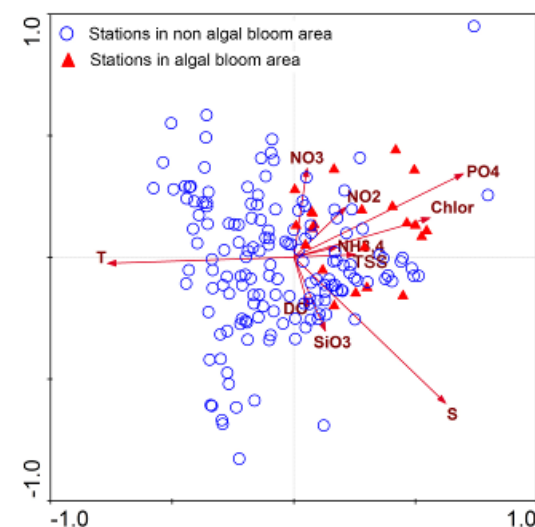


Figure 6. The sample-environmental variables biplot with algal bloom station classification

### Effects of algal bloom on fish larvae

In 2007 phenomenal blooms of toxic algae were recorded during two periods: August 2007

in the area of Vinh Hao, Phan Ri, and Phan Thiet; and September 2007 at stations DS 5 and 13 in Phan Thiet Bay. In 2008 blooms were recorded in July in the area of Vinh Hao (stations 3, 4), Phan Ri (station 8), Phan Thiet (stations 16, DS 5) and Ham Tan (stations 17, 18, 19 and 20). Our analysis showed that stations with extensive algal blooms influenced fish larvae in similar ways, forming coherent groups in our biplot. The variables  $\text{PO}_4\text{-P}$ ,  $\text{SiO}_3\text{-Si}$ ,  $\text{NH}_{3,4}\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$  and salinity have an important role during the months of blooms, but in the months when algal blooms were not observed, only temperature was a significant factor (fig. 6).

## DISCUSSION

Demineralization of the bottom sediments in the study area contributed to an increase in nutrient availability, with the highest phosphate concentrations always occurring at the bottom (Pham, 2013). As a result, algal blooms are most likely to happen during the strongest upwelling activity, when a large amount of the nutrients from the deep water are injected into surface waters creating favorable conditions to stimulate algal growth and reproduction.

The relatively low density of fish larvae in July and August may be due to increased larval mortality from algal blooms. As can be seen in our results, algal blooms occur in coastal stations along the south of the Phan Thiet Bay, and in August, 2007 dense algal concentrations were detected at these stations. In July, 2008, algal blooms were observed in coastal areas, and the larvae offshore exhibited reduced density. In individuals that were in the embryonic stage of development, algal blooms during these critical periods caused mortality of larvae, and the impact of the environmental factors affected their ability to survive (Browman 1989). The impact of toxins on fish at early stages of development has been well documented (e.g. Lefebvre et al., 2007; Lefebvre et al., 2004; Mortensen, 1985). Blooms of toxic algae produce toxins that can cause poisoning and death of fish larvae, which are very sensitive to environmental stresses.

The waning stages of the blooms can also cause changes in the environmental factors that are detrimental to the successful survival of the fish larvae. The abundance of fish year-classes is determined by food availability during the critical period of larval development (Dahlberg (1979; Platt et al., 2003). Deaths of wild and cultured fish can be divided into three categories according to three negative effects, including oxygen depletion, mechanical injury of gill tissue and toxin action (Bruslé, 1995). According to Doan et al. (2010), algal blooms dominated by *Phaeocystis globosa* occurred in waters of Binh Thuan in 2002, 2005, and 2006. Surveys in 2007 and 2008 observed *Phaeocystis globosa* blooms in September, 2007 and August, 2008. Furthermore, in August and September of 2007 and in July, August, September of 2008, blooms of the dinoflagellate *Noctiluca scintillans* (green type) occurred, reaching high densities after the *Phaeocystis globosa* blooms. Although *Noctiluca scintillans* is considered to be non-toxic and to pose no risk of poisoning to humans, dense concentrations have been observed to injure and kill fish (D'Silva et al., 2012; Escalera et al., 2007; Gopakumar et al., 2009; Thangaraja et al., 2007).

Harmful algal blooms (HABs) can produce a number of neurotoxins, such as domoic acid (DA), brevetoxin (PbTx-2) and saxitoxin (STX). Experiments on killifish (*Fundulus heteroclitus*) showed that c-Fos expression significantly increased in the anterior optic tectum of DA exposed fish compared to controls, but did not significantly increase in PbTx-2-exposed fish. In contrast to the other stressors, fish exposed to increasing concentrations of STX displayed significant decreases in c-Fos expression in the anterior and posterior optic lobes (Salierno et al., 2006). The juveniles of sea bream died after 4-10 minutes of exposure to a neurotoxic solution of three species of the red tide algae (Onoue and Nozawa, 1989). The impact of brevetoxin (PbTx-2) from *Gymnodinium breve* on the development of *Oryzias latipes* embryos was substantial; concentrations of brevetoxin from 1.0 to 3.0 ppm affect spinal development,

concentrations from 3.1 - 3.4 ppm affect the brain, and concentrations from 3.4 to 4.0 ppm affect optical development. Eggs hatched normally at doses less than 2.0 ppm. A saxitoxin concentration of 4.1 ppm led to complete cessation of embryonic development in experimental conditions (Kimm-Brinson and Ramsdell, 2001). Lefebvre et al. (2004) found that the toxic STX of *Alexandrium catenella* can impact the neurological function of larvae and juveniles of *Clupea harengus pallasii* and found that larvae were more sensitive to lower STX levels.

Other environmental factors also play a critical role in the development of larval fish. Dahlberg (1979) suggested that reduced oxygen availability in the water can be the cause of death for the fish larvae. According to Bruslé (1995), the lethal effects on finfish of harmful algal blooms can be attributed in some cases to the deoxygenation of the water when blooms decay, while in others, there is clear evidence of the involvement of biotoxins. There is either a direct action of the algae on the fish themselves, especially on sensitive organs such as the gills, liver, and tissue of the nervous system, or indirect impacts on food web that lead to mortality of predators which consume herbivores that fed on blooms. Larvae and early post larvae were highly vulnerable when exposed directly to the toxin produced by the dinoflagellate *Protogonyaulax tamarisensis* (Gosselin et al., 1989). The impact of extracted toxin of *Alexandrium minutum* on oxygen consumption rates or critical oxygen demand of milkfish (*Chanos chanos*) fingerlings has also been reported (Chen and Chou, 2001).

Although negative effects of *Phaeocystis globosa* on fish larvae have not been directly demonstrated, lethal impacts on cod larvae by a similar species (*Phaeocystis pouchetii*) have been reported (Aanesen et al., 1998). The production of toxins from the cells during bloom outbreaks and reduced oxygen in the water can cause a decline in the abundance and diversity of fish larvae in presence of algal blooms.

In the area surveyed, harmful algal blooms

(HABs) often occur in the months from July to October of the year. We focused on the environmental factors most closely related to the occurrence of HABs because of their adverse impact on fish larvae. Our analysis showed that environmental factors affecting populations of fish larvae are relatively similar in the presence of HABs but is different when no blooms form. CCA analysis also showed a marked change in the composition of fish larvae from time to time throughout the study area.

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## SỰ BIẾN ĐỔI CÁC YẾU TỐ MÔI TRƯỜNG VÀ ẢNH HƯỞNG CỦA TẢO NỖ HOA ĐẾN CÁ CON Ở VÙNG BIỂN NAM TRUNG BỘ, VIỆT NAM

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### TÓM TẮT

Sử dụng phương pháp phân tích đa biến để đánh giá tác động của sự biến đổi của các yếu tố nhiệt độ, độ mặn, hàm lượng oxy hòa tan, tổng chất lơ lửng, nitric tổng số, nitrat tổng số, a-môn tổng số, photphat tổng số, silicat tổng số, hàm lượng chlorophyll và hiện tượng tảo nở hoa đến nguồn giống tự nhiên của cá ở vùng biển Bình Thuận. Kết quả cho thấy có sự tương quan của sự biến động các yếu tố môi trường theo thời gian, không gian với thành phần cá bột và cá con. Tại các khu vực và thời điểm có hiện tượng tảo nở hoa xảy ra; cá bột và cá con giảm độ phong phú và đa dạng.

Có 5 yếu tố môi trường là nhiệt độ, độ mặn, nitrit tổng số ( $\text{NO}_2\text{-N}$ ), photphat tổng số ( $\text{PO}_4\text{-P}$ ) và silicat tổng số ( $\text{SiO}_3\text{-Si}$ ) tác động lên thành phần cá bột, cá con có ý nghĩa thống kê, các yếu tố còn lại ảnh hưởng yếu hơn và không có ý nghĩa thống kê. Vào các tháng có tảo nở hoa, các yếu tố photphat tổng số ( $\text{PO}_4\text{-P}$ ), silicat tổng số ( $\text{SiO}_3\text{-Si}$ ), a-môn tổng số ( $\text{NH}_3\text{-4}$ ), nitrit tổng số ( $\text{NO}_2\text{-N}$ ), nitrat tổng số ( $\text{NO}_3\text{-N}$ ) và độ mặn (S) có vai trò quan trọng, trong khi đó nhiệt độ (T) có tác động chủ yếu vào các tháng không có tảo nở hoa. Sự bùng phát sinh khối tảo gây nên hiện tượng tảo nở hoa đã gây chết cá bột và cá con, dẫn đến sự suy giảm mức phong phú và đa dạng của nguồn giống cá tự nhiên ở vùng biển này. Điều này sẽ ảnh hưởng đến quá trình bổ sung và phục hồi nguồn lợi cá trong khu vực.

*Từ khóa:* Cá con, các yếu tố môi trường, tảo nở hoa, ven biển Nam Trung bộ, Việt Nam.